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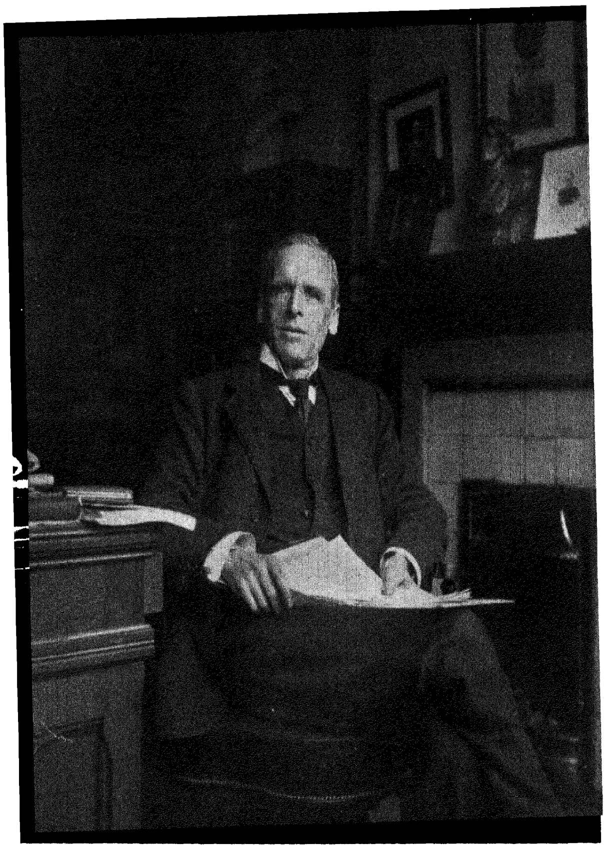

ERNEST H. STARLING, C.M.G., F.R.S.
Born 1866. Died 1927.

(Photograph by Professor H. S. Raper.)

STARLING'S

PRINCIPLES OF HUMAN PHYSIOLOGY

EIGHTH EDITION

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WITH 673 ILLUSTRATIONS, 7 IN COLOUR

LONDON

J. & A. CHURCHILL LTD.

104 GLOUCESTER PLACE

PORTMAN SQUARE

1941

1st Edition	. . . .	1912
2nd ,,	. . . .	1915
3rd ,,	. . . .	1920
4th ,,	. . . .	1925
	Spanish Translation	
5th ,,	*Revised by C L.E*	1930
6th ,,	,, ,, ,,	1933
7th ,,	,, ,, ,,	1936
8th ,,	,, ,, ,,	1941

Printed in Great Britain

PREFACE

THE present Edition is the fourth that I have prepared of this book. The enormous and, till lately, steadily growing mass of publications that deal with Physiology and cognate subjects presents many problems to the author bold enough, or rash enough, to attempt to present a unified viewpoint of the whole subject. To be unaware of, or to ignore, important developments in any branch of the subject is as certain to attract the criticism of the expert as inclusion of excessive detail would be to repel the interest of the more general reader. The maintenance of a proper balance, though desirable, is hard to attain. Moreover, an author has, or should have, some sort of story to unfold, which should give, however tentatively, his own point of view. Starling was a man of wide vision and broad ideas which led to generalisations, and the Principles, as originally produced by him, bore the reflection of those qualities, to which I can in no way aspire. The change of editorship on the one hand, and the increasing tempo of investigation on the other hand, have made inevitable a change in that ample and leisurely atmosphere which was so attractive a feature of the earlier editions, and in the present edition I have also found it necessary to rearrange rather extensively the order of treatment of the subject. The order now adopted is more in keeping with that adopted in the courses given at University College, and will, I hope, be found acceptable elsewhere.

The most considerable change in the present Edition is in Book III, which deals with the Central Nervous System. With the exception of a page or two of Starling's original text, with which I was loath to part company, I have entirely re-written this part of the book, and I hope to have done this in such a way as to have incorporated the results of some of the more important of recent investigations. It may be thought rather precipitate thus to have brought into a text-book, which lays no claim to be an advanced treatise, results which are still fresh, and in some measure awaiting full confirmation, but I would repeat that an author should at least attempt to present the subject, as understood by him, in as continuous and complete a story as possible. The parts of the subject in question are in my opinion so important, and take so adequate a place in the composition of the picture as I see it, that I feel justified in taking so small a risk for so great an advantage.

The next most extensively revised part of the book is that dealing with reproduction, where a good deal of new matter has been added, and old matter rearranged to suit more recent views. The endocrine organs have already become so active a growing point that it is small matter for surprise that a good deal of new matter has had to be introduced there also. The same is true of the vitamins, of urinary secretion, and of those numerous parts of physiology which are mainly biochemical. Throughout there has been pruning, interstitial re-writing, and rearrangement, so that few pages of the book remain entirely unaltered since the last edition.

These numerous changes have compelled an increase in size of the text, which contains 139 pages more than the last edition, and has 118 more

figures. As a good number of the figures in the last edition have been removed, the total number of new figures is larger than this ; twenty-one of them have been specially drawn.

The outlook of the " Principles " has always been scientific rather than clinical, and I can make no pretence of being able to instruct the clinician : but as the book is mainly used by students of medicine it is only fitting that there should be reference to such clinical matters as are of general interest to the subject, and to some of those specific instances in which, as has often happened, physiological inspiration has come from the observation of clinical material. The brief statements made in such connections should not be considered as due to lack of appreciation of the value of such contributions, but to a desire to save space, since there can in my opinion be no question of indebtedness on one side or the other as between clinical medicine and the principles of physiology—the relationship is too clear to need any apologist.

As in the past, I am grateful to many friends who have helped with advice, with the supply of information, and with the provision of figures. As regards the latter, I would place first and foremost Dr. Lorente de Nó, who I have to thank for Figs. 126, 127, 131, 151–156, 206–209, 223 and 224, and next Dr. W. H. Newton, whose " Recent Advances in Physiology " I have not only freely used for information, but have also pillaged for no less than 29 figures ; Professor H. S. Gasser comes next, as providing Figs. 99, 101–103, 105, 106, 109, 157, 158 and 176 ; Professor S W. Ranson for Figs. 168, 203, 237, 430, 489, 493 and 635 ; Professor Samson Wright and the Oxford Press for Figs. 189, 200, 205, 213 and 214 ; Professor J. Gaddum and the Oxford Press for Fig. 655 ; Dr. W. V. Thorpe for Figs. 574, 582–585, and various formulæ blocks ; Professor Liddell and collaborators for Figs. 178, 179, 180 ; Professor J. F. Fulton and the Oxford Press for Figs. 204 and 221, Dr. Gordon Holmes, F.R.S., for Figs 225 to 227 ; Professor B A. McSwiney for Fig. 235 ; Professor A. N. Richards for Figs. 594 to 596 ; Professor D. H. K. Lee for Fig 616 ; Professor Himsworth for Fig. 581 ; Professor Le Gros Clark for Fig. 236 ; Professor F. R Winton for Fig. 408 ; Dr. Barris for Fig. 202 ; Dr. A. C. Frazer for Fig. 563 ; Drs. Darbyshire and Davis for Fig. 297 , Dr. McMichael for Fig 472 ; Dr R Hill for Fig. 488 ; Dr. G. Stella for Fig. 502 and Dr. Robson for Fig. 645

Book IV, the Special Senses, has again been revised for me by Professor H. Hartridge, F.R.S , to whom my grateful thanks are due.

I wish also to thank Dr. F. J. W. Roughton, F.R.S., for help with the section dealing with the carriage of carbon dioxide by the blood.

My especial thanks are due to Dr. M. Grace Eggleton, D.Sc., for the preparation of the index, for correction of errors in the proofs, and for many useful suggestions.

The printers, as in the past, have again dealt with what must have been an unusually tantalising manuscript, and set it into type with an accuracy greater than could have been expected, and certainly greater than I have deserved. To the staff of Messrs. J. & A. Churchill, and in particular to Mr. John Rivers, and to their artists, my standing debt is still further augmented, for their patient and valuable help, which has been as prompt and unstinted as in the past.

C. LOVATT EVANS.

University College, London.

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SOME UNITS AND CONSTANTS

Prefixes used in nomenclature, in addition to usual ones :—

Micro = 10^{-6} *i.e.*, one-millionth (μ).
Milli = 10^{-3} *i.e.*, one-thousandth, etc.
Kilo = 10^{3} *i.e.*, one thousand.
Mega = 10^{6} *i.e.*, one million, etc.
E.g., 1 Kilo Calorie = 1 Calorie = 10^{3} calories.
1 Micro calorie = 10^{-6} calories.

The following abbreviations and signs are employed for units :

kilometre	km.	milligram	mg.
metre	m.	microgram (= 0·001 mg.)	μg. (or γ)
centimetre	cm.	kilogram-metre	kg. m.
millimetre	mm.	gram-centimetre	g. cm.
micron (10^{-3} mm.)	μ	litre	l.
millimicron (10^{-6} mm.)	mμ.	millilitre (= 1·000027 c.c.)	ml.
millionth micron (10^{-9} mm.)	$\mu\mu$.	cubic centimetre	c.c.
Ångstrom unit (10^{-7} mm. or 100 $\mu\mu$.)	A.U.	cubic millimetre	c. mm.
microfarad	μF.	minute	min.
kilogram	kg.	second	sec.
gram	g.	millisecond (0 001 sec.)	msec. (or σ)
centigram	cg.	microsecond (0·001 msec.)	μsec.

1 farad (F) = an electrical capacity which holds 1 coulomb when charged to 1 volt.

$$1 \text{ cal.} \begin{cases} = \text{heat to raise 1 gram of water through } 1^\circ \text{ C.} \\ = 4{\cdot}2 \times 10^{7} \text{ ergs} = 42{\cdot}7 \times 10^{-3} \text{ gram cms.} \end{cases}$$

1 micro cal. = 42 ergs = $42{\cdot}7 \times 10^{-3}$ gram cms.
1 Cal (or 1 kilo Cal.) = 1000 cal.

Kinetic C.G.S. unit of force = 1 dyne = force which, acting on mass of 1 gram, causes it to move with an acceleration of 1 cm. per second, per second.

Gravitational unit of force = 1 gram weight = 981·5 dynes.

Kinetic unit of work = 1 erg = 1 dyne-cm. = $23{\cdot}84 \times 10^{-9}$ calories = $10{\cdot}16 \times 10^{-4}$ gram cms.

Gravitational unit of work = 1 gram cm. = 981·5 ergs = $23{\cdot}4 \times 10^{-6}$ gram cal. = 23·4 micro cals.

Kinetic Unit of Power = 1 erg. per second.

1 kilowatt = 10^{3} watts = 10^{10} ergs per second.

Gravitational Unit of Power = 1 gram-cm. per second.

1 horse-power = 0·746 kw = 457 kg-m. per minute.
1 mole = 1 gram molecule = 6×10^{23} molecules.
Molar = 1 gram molecule per litre.
Millimolar = 10^{-3} gram molecules per litre.

Osmotic pressure of molar solution (undissociated) = 22·4 atmospheres.

Osmotic pressure of binary electrolyte (whose degree of dissociation = δ) in molar solution = $(1 + \delta) \times 22{\cdot}4$ atm. = $(1 + \delta) \times 22{\cdot}4 \times 760$ mm. Hg.

PHYSIOLOGY

CHAPTER I

INTRODUCTION

THE name "physiology," derived from a Greek root with the Latin equivalent *Physiologia*,* and originally connoting "natural knowledge," acquired its present significance in the nineteenth century. It now signifies the study of the phenomena presented by living organisms, the classification of these phenomena, and the recognition of their sequence and relative significance, the allocation of every function to its appropriate organ, and the study of the conditions which determine each function.

Life is the indefinable of biological science, as time and space are indefinables for the physical sciences. By this is meant that we cannot even approximately define life without the idea itself being implicit in our definition. Our business is, given living things, to study their phenomena.

The fundamental phenomena of life are essentially identical in all living organisms. The interest of mankind was, however, naturally awakened in connection with his own body, and the science, growing up as ancillary to medical studies, has always taken man as its chief type of study. Further, the choice of the higher animals in general receives justification from the fact that, whereas morphology must proceed from the lowest to the highest organisation, the science of function presents its problems in their simplest form in the most highly differentiated organisms. In the unicellular animal all the essential functions of living beings are carried out, often simultaneously, in one little speck of protoplasm. It is only when, as in the higher animals, the chief parts of the body have highly specialised functions that it becomes possible to peer into the details of these functions with some chance of understanding them.

Organisation. What are the fundamental phenomena which distinguish living things? Their distinctive feature is perhaps *organisation*, and in the higher members this organisation becomes more and more distinct. This greater complexity of organisation runs parallel with increasing range and power of adaptation, attained by the setting apart of special structures (organs) for the performance of definite functions. The parallelism between the development of function and structure justifies us in the assumption generally, though often only tacitly, made by physiologists, that the structure is the determining factor for the function. Further, as living animals show progressively increasing differentiation of structure in all their parts, and with this an increased power of adaptation of each part, so by increasingly delicate co-operation between their various parts, they display a progressive advance in *integration* which further augments the powers of the individual to adapt itself to its surroundings.

Energy Expenditure. When dealing with the higher animals, we are

* FULTON. *Yale J. Biol. Med.*, 1930, **3**, 59; *Science*, 1933, **78**, 109.

inclined to lay stress on the phenomena involving a discharge of energy. The life of a man in the ordinary sense of the term is made up of those movements which place him in relationship with his environment. For these movements, as for the maintenance of a constant body-temperature, a continual expenditure of energy is necessary. Experience teaches us that these movements come to an end in the absence of food or of oxygen, and that an increased call on the energies of the body must always be met by an increased demand for air and food. Two further processes must therefore be included among those making up our conception of life, viz. the function of assimilation (the taking in and digestion of food), and the function of respiration, by which oxygen is absorbed and carbon dioxide is excreted into the surrounding atmosphere.

The substances which make up our foodstuffs are all capable of oxidation. In this process of oxidation there is liberation of heat. In the body a similar oxidation occurs, and energy is thus set free which is available for the activities of the living organism.

Before we can make any accurate investigations of the conditions which determine these activities, we must know whether the laws of the conservation of mass and the conservation of energy, hold good for the processes within the living body. Thousands of experiments have been made, both on man and on animals, in which the total income of the body, viz. food and oxygen, has been weighed, and compared with the total output, viz. carbon dioxide, water, and urea, &c. In every case complete equality has been obtained.

In the determination of the equation in the case of the total energy of the body we have, in the first place, to measure the material income and output of the body, and to determine the total heat which would be evolved by the oxidation of the foodstuffs ingested to the carbon dioxide, water, &c., that are given out. We must then compare the figure so obtained with the *actual* output of heat by placing the animal in a specially constructed calorimeter, described in Chapter XLI. Rubner * thus measured in dogs the energy income and output of the body, and more elaborate experiments were made on men by Atwater and Benedict, a summary of whose results are given below :

INCOME AND OUTGO IN MAN DURING 143 DAYS

(Atwater and Benedict) †

Number of subjects used	Condition	Total number of days of experiment	Average daily net income (calculated potential energy of material oxidized) Calories	Average daily net outgo (energy given off by body) Calories.	Percentage difference
4	Rest	41	2,246	2,246	0 0
3	Work	66	4,682	4,676	− 0·1
3	Special diet	26	2,290	2,305	+ 0·7
2	Special diet and work	10	3,719	3,702	− 0·5

* RUBNER. *Ztschr. f. Biol.*, 1894, **30**, 73. Gesetze des Energieverbrauchs bei der Ernährung. (Deuticke, 1902.)

† ATWATER and BENEDICT. *U.S. Dept. Agricult. Bull., No.* **136**, 1903, p. 194.

The important deduction to be drawn from these observations is that the foodstuffs which are oxidised in the body develop in this process exactly the same amount of energy as when they are burnt outside the body.

From one aspect, therefore, the animal body may be looked upon as a machine for the transformation of the potential energy of the foodstuffs into kinetic energy, represented by the warmth and movements of the body as well as by other physical changes. In the living organism, however, it is not easy to distinguish between the source of energy and the machinery. When we endeavour to trace the foodstuffs after their entry into the body, we lose sight of them at the point where they apparently form an integral part of the living tissues. During activity there is a discharge of the products of oxidation of this living matter, which therefore becomes reduced in mass. This disintegration of the living matter, associated with activity, is always followed by a period of increased integration by the assimilation of more food. Our conception of life must therefore involve the idea of a constantly recurring cycle of processes, one of *anabolism*, repair, or integration, and the other, associated with activity, of *katabolism* or disintegration.

Growth, Reproduction. If the former process predominates, we obtain growth.

Closely associated with growth is the power possessed by all living organisms of repair, *i.e.* the replacement by newly formed healthy material of parts which have been damaged.

The process of growth does not, in the individual, proceed indefinitely. At a certain stage in its life every organism divides, and part of its substance is thrown off to form one or more new individuals, each of them endowed with the same properties as the parent organism. In the unicellular organisms, growth and division may go on until brought to an end by some change in the environment. In all the higher forms, however, the parent organism begins to undergo decay, until finally death takes place.

Adaptation. All these phenomena, viz. organisation, assimilation, respiration, the discharge of energy, growth, reproduction, and death itself, are bound up in our conception of life. All have one feature in common, viz., they are subject to the statement that every living organism is endowed with the power of *adaptation.* Adaptation is "the continuous adjustment of internal relations to external relations." A living organism may be regarded as a highly unstable system which tends to undergo disintegration as a result of any variation in its average environment. It is evident that the sole condition for the survival of the organism is that any such tendency to disintegration shall result in so modifying the relation of the system to the environment that it is once more restored to the average in which assimilation can be resumed. Every activity in a living being must be not only a necessary sequence of some antecedent change in its environment, but must be so *adapted* to this change as to tend to its neutralisation, and so to the survival of the organism. This is what is meant by adaptation.

We have no knowledge of how living things first came into existence, but having once arisen in a primitive form, the 'adaptation,' *i.e.* the reactions of the primitive living material to changes in its environment, must become ever more and more complex, since only by means of increasing variety of reaction is it possible to provide for the stability of the system within greater and greater range of external conditions. The difference between higher and lower forms is therefore one of complexity of reaction, or of range of adaptation.

Organism and Environment. Though it is a philosophical point on which there is no need to dwell, it should be emphasised that organism and environ-

ment must be viewed as a whole. But so far as possible the organism adjusts itself to any changes in its environment or "adjusts its internal to its external relations."

This "adjustment of internal to external relations" is possible only within strictly defined limits, limits which increase in extent with rise in the type of organism, and in the complexity of its powers of reaction. Among the chief of them are temperature, and the presence of food material, water and oxygen. Many organisms are killed by the alteration of only a few degrees in the temperature of their environment. In the higher animals a greater stability in face of such changes has been accomplished by the development of a heat-regulating mechanism, so that, provided sufficient food is available, the temperature of the body is maintained at a constant level. In the primitive condition, the food material must be of a given character and form a constant constituent of the surrounding medium. In the higher forms, the development of a complex digestive system has enabled the organism to utilise many different kinds of food, while the storage of excess as reserve material, provides for a constant supply of food to the cells of the body, even when it is temporarily wanting in the environment. Moreover, but few cells of the higher organisms are called upon to face the vicissitudes of light, temperature, chemical composition, etc., of the general environment: instead, most of the cells of the body live in darkness and in contact only with that inner environment, the blood and tissue fluid, the composition and temperature of which are kept constant by wonderfully adapted arrangements. The name *homeostasis* (homoiostasis) is given by Cannon to the condition of uniformity which results from the responses of living things to changes in their environment.

To sum up. Our objects in the study of physiology include the description of the chief reactions of the body to changes in its environment, the analysis of these reactions into the simpler reactions of which they are made up, and the assignment to each differentiated structure of the organism its part in every reaction. We must determine the conditions under which each response takes place, so that we may learn to evoke it by application of the appropriate change of environment.

A response involves expenditure of energy, derived from chemical change in the reacting organ, and ultimately from the disintegration or oxidation of the foodstuffs. Our next task must therefore be the analysis of the changes, with a view to determining the whole sequence of events, from the occurrence of the external exciting change to the finished reaction. In short, it is the office of physiology to discover the sequence of events in the living organism under all manner of conditions. In attacking this problem our methods cannot differ fundamentally from those of the physicist and chemist. In every case our experiments will consist in the observation and measurement of processes of one kind or another which we shall interpret in terms of mass or energy. Physiology, if it could be completed, would therefore describe the *how* of every process in the body. It would state the sequence of events and would summarise these as so-called 'laws.' These laws would, however, no more explain the phenomena of life than does the 'law of gravitation' explain the fact that two masses attract each other with forces directly proportional to the product of their masses. Nor can we hope to explain physiological phenomena by reference to the laws of physics and chemistry, since these themselves are only expressions of sequences, and not explanations. With every growth in science, however, its generalisations become wider and its laws summarise ever more extensive groups of phenomena. The principle of adaptation is the only formula

which will include all the phenomena of living beings. This principle must provisionally be accepted as fundamental by the biologist, as the physicist accepts the first law of thermodynamics.

The consciousness of the inadequacy of our explanations, which must be experienced with greater force the more deeply the physiologist endeavours to peer into the processes within the living cells, has led some to the assumption of a special quality in living organisms which is designated as 'vital activity.' Such views are classified together under the term '*vitalism.*' As a working hypothesis it must be sterile because there is no evidence that the introduction of vitality enables the known laws of nature to be overruled in any way: whatever the living thing accomplishes, it may do so with the aid of quite orthodox phenomena. That the presence of life does, however, result in an orderly marshalling of these orthodox phenomena, and that in a quite peculiar way, could hardly be questioned. This again, is a philosophical point and need not detain us further than to note the apt remark of Claude Bernard, that "vital force directs phenomena which it does not produce; physical agencies produce (in living things) phenomena which they do not direct." In many cases, however, the terms '*vitalism*' and its antithesis '*mechanism*' are used unjustifiably. Because the phenomena of living matter are not arbitrary, and since the conditions are infinitely complicated, they are incapable of arbitrary simplification when compared with those concerned with the study of non-living matter, and are hence more difficult to control. The physiologist, basing his belief in the principle of adaptation must reject vitalistic "explanations" and seek to discover by experiment the conditions which determine the appearance of phenomena in living things. But he must equally reject an arrogant and superficial doctrine of "mechanism" which would suppose that life can be the outcome of any chance encounter of physical and chemical phenomena, and here again the principle of adaptation must be his guide in refusing to accept the suggestion that physiology is nothing more than applied chemistry and physics. Holding this principle always before him, he must abide by the answer he receives from his experiments as to the ways and means of adaptation, and can hope for little more.

Throughout this chapter we have admitted no necessary dividing line between the different classes of phenomena in the conceptual universe, although in the present state of our knowledge we are far from being able to include the whole of them under the same general laws. It might be objected that in taking up this attitude we have left out of account one supreme fact, viz. the existence of *consciousness* in ourselves. As a comparative and objective study, however, physiology is concerned, not with the study of consciousness, but with the conceptions in consciousness of the *phenomena* presented by living beings.

THE METHOD OF PHYSIOLOGY. Physiology derives most of its inspiration from observations incidental to the phenomena of our daily life and from those made in the course of medical practice. In the prosecution of his enquiries the physiologist often must make appeal to other sciences, but especially to chemistry and physics. In the first place the chemistry and physics of the various separate organs of the animal body must be investigated. This involves first an accurate knowledge of the gross and minute structures, and hence requires the aid of anatomy and histology. Here very frequently, too, comparative anatomy and histology furnish valuable clues to function.

Then the chemical, physical and physico-chemical build must be taken into consideration with a view to the determination of the changes which

these exhibit under altered conditions of environment, chemical or physical in nature.

In this way analytical physiology is built up, by experiments, carried out on living animals, or on separated parts of them surviving for a time under suitable conditions ; knowledge of the properties and functions of the various structural parts is obtained. Then, in synthetic physiology, we attempt to find how, by integrative processes, the functions of the body as a whole are synthesised from the co-operative activity of the various parts, and how the organism as a whole reacts, by appropriate adjustment of its individual organs, to changes in its external environment.*

" *Mechanisms* " *in Physiology.* In his physiological studies the student will often meet with the word " mechanism," *e.g.* " the mechanism of gastric secretion " ; " . . . of walking " ; " . . . of reflex action," &c. This rather overworked word need not be taken too seriously, because as a rule it has no more significance than the equally busy and now notorious adjective " marked." " Mechanism of " really means, as a rule, " the phenomena concerned in . . . " as *e.g.* " the mechanism of bone formation," though in other instances, which the reader can readily find as *e.g.* " The mechanism of speech," the word is more literally employed.

The Normal. Individuals of the same species resemble one another more or less closely in structure and in their physiological properties, and this tendency of biological characters towards a standard type is an expression of the approximate harmony between the organism and its environment. The resemblance is greatest between animals born of the same parents at the same time. As species further apart are considered the differences become greater, and, between the extremes of the animal kingdom are so great that comparisons become vague.

Yet, even when the relationships between individual animals are of the closest kind, *e.g.* in the human race, these do show differences from one another. It is a duty of human physiology to study the normal human individual, since this provides an important baseline for the study of disease ; yet it is far from easy to state what is the normal of any given character. Further, in all physiological experiments the results must show a good deal of apparently arbitrary variation depending on the individual animals used. Hence the importance of proper controls, and the decision of what constitutes a significant result, cannot be overestimated. Often, in order to be certain that representative results are being considered, it is necessary to repeat experiments many times. The error of observation which is usually slight in the exact sciences, is often great in the biological ones, on account of the variability introduced by the use of varying individuals. The results are, however, capable of being treated by the application of the same laws of probability as are employed in other sciences, *i.e.* they can be treated statistically. Caution is especially necessary in drawing conclusions from a limited number of experiments, as to whether the results obtained differ from those of a control series or not. Thus if a given result happens 4 times out of 8 in one series and 7 times out of 16 in another, we are not justified in concluding that it happens more often under the conditions of the first, because chance could easily account for the difference. But 4 out of 8 in one series and 3 out of 16 in the second would be a significant difference.

It is a common practice to speak of averages, that is, of arithmetic means, as one way of overcoming the difficulty, *e.g.* we can say that the average number of red cells per cubic millimetre of blood in a given community of

* As an example of essays in synthetic physiology may be quoted J. BARCROFT's " Features in the Architecture of Physiological Function," Cambridge, 1934.

men is 5·2 millions. Such a method, though the simplest and the one most commonly used, is often unsatisfactory, because it does not tell us how great or how relatively frequent are the departures from the mean, so that we should have no means of knowing in the example quoted whether 4·5 millions was within the normal range or not. There is consequently a growing practice of using more accurate means for the expression of statistical results.

One of the methods employed is the use of the *frequency diagram*, an example of which is given in Fig. 1, which illustrates data regarding the stature of a large number of individuals. These have been divided into frequency groups differing in stature by 1 inch. The height of each rectangle is proportional to the number of individuals having the height shown at the abscissa, the grouped figure being called a histogram. A smoothed curve, or frequency curve, drawn from this, shows the frequency with which any stature between the given limits occurs. (Other diagrams are given in Chapter XXIX showing distributions for blood counts.)

The *standard deviation* or mean error, is one of the means of improving the value of results ; it measures the amount of variation in the individual figures from which the

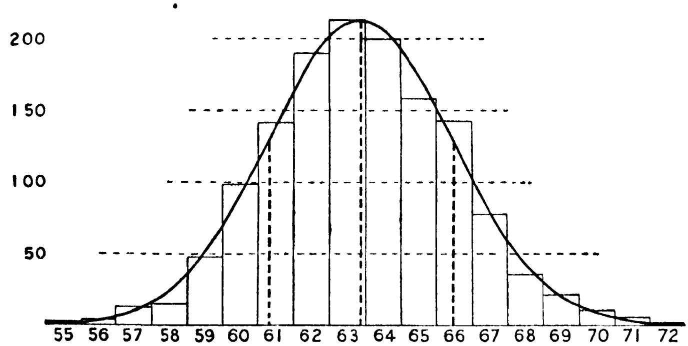

FIG. 1. Frequency diagram of distribution of stature of 1,375 women, in groups differing by one inch (Pearson and Lee.) (From Fisher's "Statistical Methods for Research Workers." London Oliver & Boyd)

average was obtained. The accuracy of an average result depends on the square root of the number of observations made, so that, in a series of n determinations with individual differences summed up as Σd, it can be shown that the standard deviation of a single observation is $\pm \sqrt{\frac{\Sigma d^2}{n-1}}$, while the standard deviation of the average is given by the formula $\pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

To take an illustration given by Burn, we may consider the investigation of the lethal dose of a drug, as determined on a series of five cats, which gave the result as 17·44 ± 1 67 c.c. The figure 1·67 is the standard deviation of the average, and means that, " if a number of determinations of the average lethal dose be made, each time on five cats, then twice out of three times the difference between the value obtained and the true value will be less than 1·67, and once out of three times this difference will be greater than 1 67." If we take twice the standard deviation, and call the result 17·44 ± 3·34 c.c. the report will probably be wrong only once in twenty-two times ; or if three times the S.D. or 17·44 ± 5·01 c.c., then it will probably only be wrong once in 370 times.

A difference between two series of similar experiments with standard deviation = σ may be considered to be significant if, the two series having given results of $x_1 \pm \sigma_1$, and $x_2 \pm \sigma_2$, the ratio $\frac{x_1 - x_2}{\sqrt{\sigma_1^2 + \sigma_2^2}}$ is greater than 3.

The *probable error* of a mean is given by the formula $\pm\ 0{\cdot}6745\ \frac{\sqrt{\Sigma d^2}}{n\,(n-1)}$ or, by the more convenient approximation of Peters, the probable error of the arithmetic mean of a series of observations $= \pm \frac{0{\cdot}8455\ \Sigma\,(+\,d)}{n\,\sqrt{(n-1)}}$ where $\Sigma\ (+\,d)$ is the sum of the deviations of every observation from the mean, their sign being disregarded. The probable error determines the degree of confidence we may have in using the mean as the best representative value of a series.*

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History of Physiology

As it is impossible to realise the present position of any science without some knowledge of the history of its development, the serious student of physiology should acquaint himself with the outlines of its history. The following works are recommended for this purpose :—

FOSTER (1901). Lectures on the History of Physiology. (Cambr. Univ. Press.)
FRANKLIN (1933). A Short History of Physiology. (John Bale.)
FULTON (1930). Selected Readings in the History of Physiology. (Springfield : Thomas.)
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* For further information on this specialised subject, see the book by FISHER and the articles by BURN and DUNN. A brief account is given in LAMB's " Introduction to Human Experimental Physiology." (Longmans, Green, 1930.)

BOOK I

GENERAL PRINCIPLES

A. Structural Principles

B. Biochemical Principles

C. Biophysical Principles

A. STRUCTURAL PRINCIPLES

CHAPTER II

THE STRUCTURAL BASIS OF THE BODY*

THE CELL

SINCE the formulation of the *cell theory* of life by Schwann and Schleiden about 1840, it has been customary to regard the function of any organ, or indeed of the whole body, as the sum of the functions of its constituent cells. The cell is therefore a physiological as well as structural unit.

Many of the lower forms consist only of a single cell. In this cell are represented all the adapted reactions which we associate with life. Every one of the higher organisms in its development commences with an unicellular stage, namely, the fertilised ovum.

The general characteristics of cells present important similarities, whether we are considering a cell which forms the whole of an organism or a cell which is but an infinitesimal part of a highly developed animal.

A cell is a small mass of protoplasm containing a nucleus. In the multicellular animals there are cells of many different kinds of structure and function. In this way there is a division of labour. The organism, biologically, is something more than the mere sum of its component cells, since it directs the growth and functions of these to its own ends, just as a nation is, biologically, something more than a large number of individuals. We must regard the organism also as a biological unit of definite significance.

Every cell, so far as we have been able to ascertain, is derived from some pre-existing cell, by a process of division, and every nucleus similarly from a parent nucleus. These important laws, so significant in the study of heredity, were expressed by Virchow (1855) and Flemming (1882) in the dicta "*omnis cellula e cellula*" and "*omnis nucleus e nucleo*" respectively. As a necessary consequence it follows that the cytoplasm of the cell is also derived from the previously existing cytoplasm of the cell from which it arose.

Since we shall have to use the term 'protoplasm' on many occasions in the course of this work, we must have a definite conception of what we mean by it. When employed by physiologists it generally implies any material which we can, on a study of its behaviour to changes in its environment, regard as endowed with life. Though it may be convenient to have a word such as protoplasm signifying simply 'living material,' it is important to remember that there is no such thing as a single substance—protoplasm. The reactions of every cell as well as its organisation are the resultant of the molecular structure of the matter of which it is built up. The gross methods of the chemist show him that the composition of the 'protoplasm' of the muscle cell is entirely different from that of a leucocyte or white

* The matter of this chapter will be familiar to students who have studied Histology, Cytology, and Embryology, and only gives such broad outlines as to recall the importance of those subjects to Physiology.

blood corpuscle. The finer methods of the physiologist show him that every sort of cell in the body has its own peculiarities of reaction to changes in its surroundings, and the reactions of the whole organism are but the sum of the reactions of its constituent cells. There is not *one* protoplasm therefore, but an infinity of protoplasms.

Even in a single cell there is more than one kind of protoplasm. In its chemical characters, in its mode of life, and in its reactions, the nucleus differs widely from the cytoplasm. Both are necessary for the life of the cell and both must be considered, according to our present ideas, as 'living.' In the cytoplasm itself we find structures or substances which we must regard as on their way to protoplasm or as products of the breakdown of protoplasm (Fig. 2); but in many cases it is impossible to say whether a given material is to be regarded as lifeless or living. Even in a single cell we may have differentiation among its different parts, one part serving for the process of digestion while others are employed for the purpose of locomotion. It will be clear also that, while it is convenient to regard the cell as the functional and structural unit of living things, this view is open to criticism on the ground that portions cut off from cells may still show evidence of being alive. When looked at from this standpoint it is not

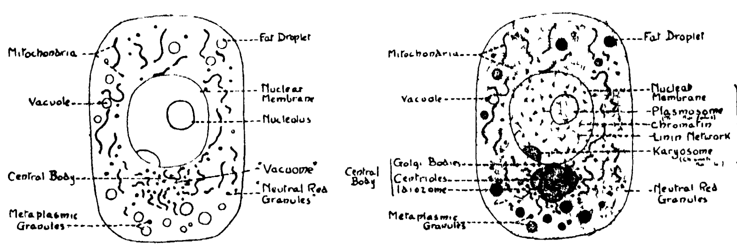

FIG. 2. Diagram to show contents of typical cell. On left, structures which can be seen under appropriate conditions in the living cell. On right, the structures demonstrable by various fixing and staining methods. (LUDFORD.)

possible to indicate any clear-cut unit—the whole of the protoplasm is alive.

VITAL PHENOMENA OF CELLS. A. *Assimilation*. The activity of every living being can be regarded as compounded of two phases, *assimilation* and *dissimilation*. By assimilation we mean the building up of the living substance with material obtained from the external world. In this process substances of high potential energy are formed, and this energy is obtained either from that imparted to the system at the moment of assimilation, as *e.g.* in the assimilation of carbon from carbon dioxide under the influence of the sun's rays, or from that contained in the foodstuffs themselves. In animal cells it is the latter method which is adopted and a foodstuff therefore connotes some substance which can be taken in by the cell and can serve it as a source of chemical energy. The energy required for the movements and other vital activities of the cell is derived from a disintegration or dissimilation of the protoplasm, generally associated with the process of oxidation. In assimilation, besides the building up of living protoplasm, there may also be a synthesis of more complex from less complex compounds, without their necessary entry into the structure of the living molecule. In the absence of any definite criteria by which we may judge as to the living or non-living condition of parts of the cell, it is a little dangerous to draw any hard-and-fast distinction between these

two sets of processes. Assimilation requires the ingestion of food into the organism, and then its digestion, *i.e.* its solution in the juices of the cells. These two processes are succeeded, through stages which we cannot trace, by an actual growth in the living material. Digestion is apparently effected in most cases by the production of solutions containing *enzymes*, *i.e.* agents which have the power of hydrolysing the different foodstuffs and thus rendering them soluble.

In the vast majority of living organisms the energy for their activities is derived from oxidation. A necessary condition therefore for the life of these cells is the presence of oxygen. As a result of this oxidation, products are formed which are of no further value to the cell and are therefore *excreted*, *i.e.* turned out of the cell. The chief of these are the products of oxidation of carbon and hydrogen, namely, carbon dioxide and water. There are also many substances resulting from the oxidation of the nitrogenous portions of the protoplasm which have to be excreted in the solid or dissolved form.

Although the assimilation of oxygen is so general a quality of living protoplasm, the presence of this gas does not seem to be necessary for all kinds of life. Indeed, most cells can survive for a short time in its absence, and can even carry out oxidative processes by indirect reactions. Further, many bacteria are known which are anaerobic, *i.e.* exist only in the absence of oxygen. Examples of such are bacillus tetanus and the bacillus of malignant œdema.

B. *The Phenomena of Dissimilation.* The activities of a living cell or organism can be regarded in every case as dependent originally on environmental change, and are adapted to this change, *i.e.* are of such a nature that they tend to preserve the organism intact. The property of reacting in such a manner to changes in the environment is fundamental to all protoplasm and is spoken of as *excitability*, and any change which will influence an organism and cause a corresponding adaptive change in it is known as a *stimulus*. Stimuli may be of various kinds. Thus mechanical, thermal, chemical, electrical changes, light and so on may act as stimuli. The reactions which they evoke in every case involve chemical changes in the protoplasm, *i.e.* changes in the metabolism of the cell. Sometimes this change may be assimilatory in character, leading to an increased growth of the protoplasm, or at any rate to a cessation of dissimilation. In such a case the stimulus is spoken of as inhibitory, because it diminishes or prevents the output of energy by the organism. The frequent result of a stimulus is an increased output of energy, which may appear in the form of movement, heat, or chemical change.

A common feature of all dissimilatory changes evoked by the application of a stimulus is that the energy of the reaction is many times greater than the energy represented by the stimulus, the excess, of course, being supplied at the expense of the potential energy of the material of the living protoplasm. This disproportion between stimulus and reaction can be well illustrated on an excitable tissue such as muscle. Thus in one experiment the gastrocnemius muscle of a frog was loaded with a weight of 48 grammes. The nerve running to the muscle was placed on a hard surface and a weight of half a gramme was allowed to fall upon it from a height of 10 mm. The muscle contracted in response to this mechanical stimulus applied to the nerve and raised the weight 3·8 mm. In this case the work performed by the muscle was $48 \times 3{\cdot}8 = 182{\cdot}4$ gramme-mm., while the potential energy of the stimulus represented only $0{\cdot}5 \times 10{\cdot}0 = 5{\cdot}0$ gramme-mm. Thus the work performed by the muscle was thirty-six times larger than the energy of the stimulus applied to the nerve.

In the case of unicellular organisms and single cells, definite classes of motor reaction to stimulus have been described. Among the primitive reactions of cells perhaps the most important in the life of higher animals are those grouped under the term *chemiotaxis*. In the fertilisation of the ovum in the prothallus of ferns by the antherozoids it was shown by Pfeffer that the movement of the antherozoids towards the ova is effected in response to a chemical stimulus, probably malic acid. The movements of the white corpuscles of the blood of the higher animals are also largely determined by their chemical sensibility, and various substances can be divided into (*a*) those which exercise positive and (*b*) those which exercise negative chemiotactic influence on the leucocytes. Thus the introduction under the skin of an animal of a capillary tube containing a solution of substances of the first class, such as peptone, tissue extracts or the chemical products of certain bacteria, leads to an accumulation within the tube of leucocytes which pass to it from all the surrounding tissues. Other substances, such as quinine, exert a negative chemiotaxis. Tubes filled with these, after introduction into the subcutaneous tissue of a mammal, will be found many hours later to contain no leucocytes at all.

METHODS EMPLOYED IN THE STUDY OF MINUTE STRUCTURE. The study of structure for its own sake properly belongs to the subject of anatomy and histology, but the relations between structure and function are so intimate that the science of physiology would be incomplete if it did not take considerations of structure into account. Moreover, there has grown up recently an important branch

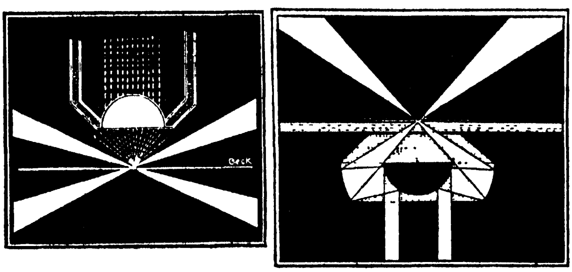

FIG. 3. To illustrate the principle of dark-ground illumination, and the method by which this is obtained with the use of a special substage condenser. (Courtesy of Messrs. R. and J. BECK.)

of physiological investigation, namely, the enquiry into the physiology of individual cells, in which methods of histological research are in constant use.

With the assistance of the microscope and the customary histological technique for preparation of material, the structure of all the organs and tissues of the body has been thoroughly explored. This histological technique, however, has its limitations. We are often in doubt as to how far the structures we see in fixed and stained preparations have any real existence in the living cells. A method of fixation which produces minimal change of structure and physico-chemical arrangement is to dry the tissue while in a frozen state.* A further difficulty is that the power of the microscope to reveal minute details has been developed to the uttermost limit, namely, to enable us to see structures whose dimensions are of the same order as the wave-length of light. Some further resolution can be got by using light of shorter wave-lengths ; more detail will be seen with green illumination than with yellow light, and by photomicrography with invisible ultra-violet light of wave-length 3,000 A.U. still more details can be unfolded. A further result of using ultra-violet light has been to show that certain structures, *e.g.* chromosomes, are more opaque to it than others, though both types may be equally transparent to the visible rays ; hence not only finer structures, but actual new structures, may be revealed.

A further refinement is by the use of the principle of the ultra-microscope in what is called *dark-ground illumination*. With the aid of a paraboloid condenser the light is thrown nearly transversely across the preparation (Fig. 3), so that the field is dark except where particles lying in the path of the beam are able to reflect the light into

* BENSLEY *et al.* *Anat. Rec.*, 1933, **57**, 205, 217, 369 ; **58**, 1 ; 1934, **60**, 251, 449.

the microscope. This method will reveal the position of particles in living protoplasm, and give us some idea of their size, even though they are so small as to be invisible by any direct microscopic examination.

Much valuable information has been obtained with regard to the structure and behaviour of living cells by means of *micro-manipulation*, or micrurgical technique, which has been extensively used by Chambers as a means of dissecting the live cell or of injecting various fluids into it. The living cells are suspended, as a hanging-drop preparation, from a cover-glass, and are manipulated and dissected by means of two fine glass needles, the movements of which in all directions are controlled by micrometer adjustments (Fig. 4). In dissection, one needle is brought up so as to hold the cell or tissue while the other needle is employed to dissect it, the whole operation and its

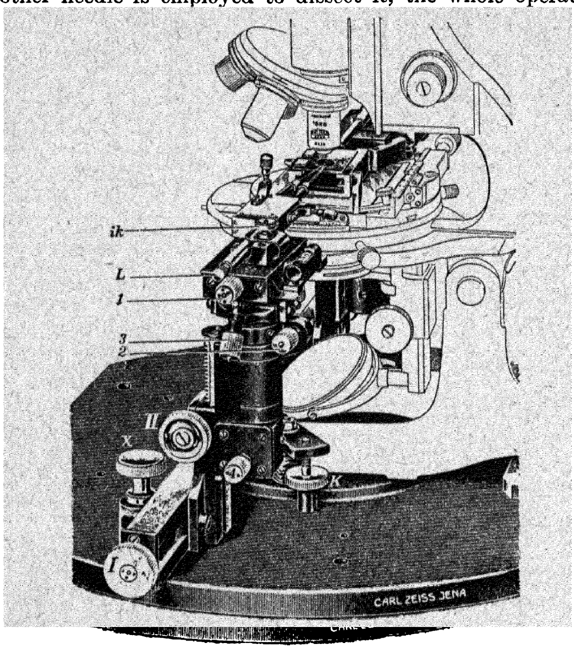

FIG. 4. Micro-manipulator in position It consists of a rigid base on which is mounted the microscope, and on either side of this an operating stand. The manipulations are in the main effected by six screws, two of which transmit coarse motions to the operating stands as a whole, while the other four screws serve to actuate the micro-instruments I Coarse perlateral screw , motion, from right to left, and *vice versa*. II. Coarse vertical screw ; motion, in an up and down direction 1. Fine perlateral screw 2 Fine sagittal screw , motion, forward and backward, and *vice versa* 3 Diagonal screw ; motion, radially up and down 4. Fine vertical screw In the figure a double needle is shown on one stand and a single one on the other. (Courtesy of Messrs. CARL ZEISS.)

results being watched under the microscope · injection is performed by fixating the cell with a needle and then perforating it at the desired place with a fine glass capillary through which the fluid is then forced from a micrometer syringe.

Another method which has found many applications of late is that of *tissue culture*, in which small portions of embryonic tissue are taken and kept at suitable temperatures in a nutrient fluid, such a mixture of embryo extract, blood plasma and saline solution. The whole preparation must be aseptic, and is usually made as a hanging drop in a chamber sealed off from the air with vaseline. The tissue continues to live, and under certain conditions the cells grow and multiply in an apparently normal manner until the cell mass reaches a certain size, when growth ceases and degeneration sets in unless a small piece of the tissue is taken and used to start another similar culture. In some cases the process of sub-culture can apparently be repeated indefinitely. Much has been learned of the structure, growth, and differentiation of embryonic cells by this method.

The histological staining methods are very usefully supplemented by a variety of so-called *intra-vitam staining* methods in which the dye is presented to the living organism, and *supra-vital staining*, in which the dye is added to a solution in which surviving tissues or isolated cells are suspended. Certain portions of cells have an affinity for particular dyes. Thus the mitochondria are stained in the live cell by Janus green, nerve material by methylene blue, many cell granules by neutral red, certain connective tissue cells by Pyrrol or Trypan blue, &c. This gives a valuable method for classifying cells, as well as for obtaining information with regard to their structure.

A good deal can be learned about the distribution of chemical substances within cells by means of various *microchemical reactions*. Thus the presence and location of fat, protein, glycogen, potassium, iron, &c., can be discovered by appropriate colour reactions applied to sections of tissues or to isolated cells. The distribution of the inorganic constituents can be studied by means of *micro-incineration* *; sections of the tissue are incinerated on the slide, and on dark-ground illumination the deposit of ash is seen to vary in density in different kinds of cell or different parts of each cell.

ULTRASTRUCTURE OF PROTOPLASM. In its last resort the study of structure must become that of molecular orientation, and hence lies beyond the reach of ordinary microscopes. The electron microscope †, giving a resolution thousands of times greater than the ordinary microscope has already found some applications, and applications of the optical properties to polarised light have also been used. By the use of X-ray diffraction methods also, information regarding the molecular components of tissues can be obtained. The methods are complicated and the interpretation of results, though promising, is still largely tentative.

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STRUCTURE OF THE CELL. The nucleus is generally bounded by a definite *nuclear membrane*. The contents of the nucleus are probably fluid in life, but on damage, death and coagulation rapidly occur. Within the nucleus are one or more smaller bodies called *nucleoli*; in the living non-dividing nucleus no other structures are usually visible. After fixation various artefacts appear in the nucleus, *e.g.* masses or filaments of a material known as *chromatin*, which are strung, so to speak, on a fine network of material known as *linin*. The material filling up the meshes of the network is the nuclear sap or *nucleoplasm*. When the cell is about to divide, the chromatin becomes aggregated into filaments called *chromosomes*, and these are visible in the living cell.

The cytoplasm, which varies greatly in extent in different cells, varies also in its appearance, especially after fixation, being sometimes homogeneous, sometimes alveolar, sometimes granular in structure. In it can often be distinguished differentiated parts which may be regarded as organs of the cell.

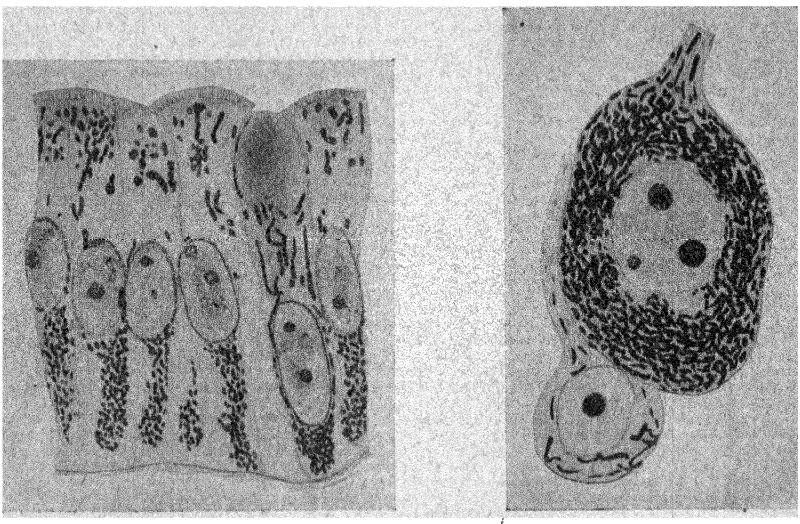
The most universal of these cell inclusions, or possibly cell organs, are the *mitochondria* or *chondriosomes*. These occur in the form of granules, rods or filaments in almost every type of cell, animal and vegetable (*cp.* Fig. 5). Their function is unknown, though they seem to be more numerous and longer in cells which are very active. Their presence must largely increase the surface of phase-boundaries within the cell, and it has been suggested that important chemical reactions occur at these surfaces of contact between mitochondria and cytoplasm. They are often grouped at one pole of the cell; thus in gland cells they are frequently found at the pole nearest to the blood capillaries and furthest from the lumen. They are more abundant in young than in old cells, and in the living cell are seen to move about readily in the cytoplasm. When the cell divides the mitochondria are shared between the two daughter cells. In some conditions the mitochondria can be dragged out of the cell by microdissection methods, hence they are relatively solid. They contain some 40 per cent. of fatty substance, and two different proteins, but are said to contain no lecithin or cephalin.‡ Usually they are dissolved by alcohol and by weak acids, and so are not displayed in cells hardened with a view to display nuclear structure. In the living cell they can be stained with Janus

* POLICARD. *Protoplasma*, 1929, **7**, 464. SCOTT. *Anat. Rec.*, 1931, **48**, 31.
† V. BORRIES and RUSKA. *Naturwiss*, 1939, **17**, 281.
‡ BENSLEY and HOERR. *Anat. Rec.*, 1934, **60**, 449

green. They are best preserved by hardening in formalin and bichromate and staining with iron hæmatoxylin.

An organ of considerable importance is the *centrosome*, a specialised area of cytoplasm often containing a more deeply staining particle, the *centriole*. In the resting, *i.e.* the non-dividing cell, it commonly lies near the nucleus, and is sometimes double; cells which are incapable of division, *e.g.* nerve cells, have no centrosome, but there may also be no centrosome in certain resting cells which are nevertheless capable on occasion of further division; in those cases, when the time for division approaches, a new centrosome is formed in the cytoplasm. The process of cell-division is apparently heralded and initiated by a preliminary division of the centriole and centrosome. Next there appear striæ radiating out from each centriole, the structure so formed being called an *aster*. The asters on microdissection behave as relatively rigid elastic bodies, very distinct from the surrounding more liquid cytoplasm, and the two asters grow rapidly in size and appear to repel one another, so that they tend to travel to opposite poles of the cell.

Another cell structure is the so-called *Golgi apparatus*, a reticular structure often located at one pole of the cell, soluble in alcohol, blackened by osmic acid and, after



A B

FIG. 5. Various Cells stained to show Mitochondria. (E. V. COWDRY.)
A. Lining Cells of the Small Intestine.
B. Two Nerve Cells, with different forms of Mitochondria.

suitable fixation, stainable with silver salts (Fig. 6). In secretory cells it is usually found between the nucleus and the lumen. The functions of the Golgi apparatus are uncertain. Its filaments are of lipoid nature, and it has been suggested that certain products of cell activity, *e.g.* zymogen granules, can be assembled at the surface of the filaments.

Special inclusions are also met with in certain cells, the best example being the Nissl bodies of nerve cells. Sometimes these structures are artefacts produced in the course of fixation. In addition to these cell organs the cytoplasm may contain visible *granules* of material of various types, which are usually formed by the activity of the cytoplasm or stored there until required. Thus we have granules of *zymogen* found in secreting cells and representing a precursor of the enzymes which the cell liberates during activity, or granules of food material provisionally stored in the cytoplasm until required, *e.g.* fat or glycogen.

THE PHYSICAL STRUCTURE OF PROTOPLASM. It is usually impossible, even under the highest powers of the microscope, to make out any structure whatsoever in living cytoplasm. It is an apparently homogeneous liquid mixture of colloids. Various inclusions and granules may, of course, be visible, but these are not essential components, and are not necessarily living. In some cases examination of a cell, even

unstained, shows some differentiation betweeen a more or less regular framework or meshwork and a more fluid portion filling up its interstices, and these appearances are the rule when cells have been fixed by hardening fluids. All the results obtained in this manner must be regarded with some suspicion, since, as has been shown by Fischer and by Hardy,* it is possible to imitate artificially the various structures, which have been assigned as characteristic of protoplasm, by hardening a homogeneous colloidal solution such as egg-white by different methods. All fixation methods have in common the production of such conditions as lead to precipitation of the cell proteins; and though by comparison of fixed preparations much valuable information is obtained, the results of the treatment cannot be held to give any picture of the actual structures present in the living cells. The results of differential staining, especially after fixation, also need further caution in their interpretation, since we know so little of the factors which combine to give any particular result. Thus various theories of protoplasmic structure, which have been put forward as the result of observations on hardened tissues, possess little more than historic interest.

Most histologists are in accord that during the life of a cell its protoplasm, as observed under the microscope, may be either structureless or may present certain structural

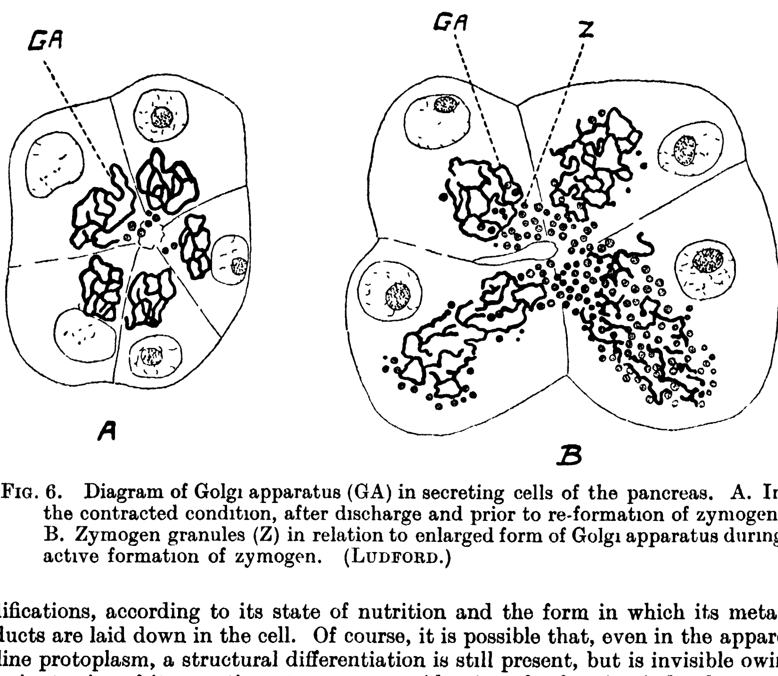


FIG. 6. Diagram of Golgi apparatus (GA) in secreting cells of the pancreas. A. In the contracted condition, after discharge and prior to re-formation of zymogen. B. Zymogen granules (Z) in relation to enlarged form of Golgi apparatus during active formation of zymogen. (LUDFORD.)

modifications, according to its state of nutrition and the form in which its metabolic products are laid down in the cell. Of course, it is possible that, even in the apparently hyaline protoplasm, a structural differentiation is still present, but is invisible owing to the minute size of its constituent parts or an identity of refractive index between the alveolar walls and their contents.

When subjected to stress, cytoplasm may show fine fibrillations, which vanish again when the stress is removed. The radiations of the aster are possibly the result of such a state of strain originating in the neighbourhood of the centrosome. They disappear at once if the cell is etherised.

Since the study of the behaviour of the cell shows that it must possess a much more complex structure than that which is revealed by the microscope, and one which permits of the spatial differentiation of the different chemical processes that may occur at one and the same time in the protoplasm, many theories of an ultramicroscopic cell structure have been put forward. Ultramicroscopic examination of living cells certainly shows that the cytoplasm is crowded with granules of various sizes, even though it may appear homogeneous to ordinary microscopic observation. These granules are able to move about within the cell, and indeed, in certain cases, display a lively

* HARDY. *J. Physiol.*, 1899, **24**, 158.

Brownian movement, *e.g.* in muscle cells, leucocytes, &c. In many animal cells, however, the cytoplasm appears to be too viscous to allow of very active movement.

One question which has been much discussed relates to the physical condition of protoplasm. Is it to be regarded as a viscous fluid or as a soft solid? The perfect potential mobility of the protoplasm of many cells, as instanced by the flow of the substance of an amœba into its pseudopodia, or the ready movements of granules or mitochondria within living cells, indicates a fluid character for the protoplasm. This view is supported by the observation that when small amounts of water are injected into the substance of a cell the water at once mixes freely with the cytoplasm. Against such a character has been urged the fact that in protoplasm we may have shape, organisation, and power of resistance to deformation—qualities which are generally associated with the possession of solidity. It must be remembered, however, that the absence of resistance to deformation which is characteristic of a liquid, applies only to the internal molecules, and that the surface of any liquid is in a condition of tension which not only limits deformation but presents considerable resistance to any enlargement of the surface. The continued existence of protoplasm in a watery environment shows that not only must its composition be different from that of its environment, but that there must be a distinct surface separating the two. The superficial layers of the protoplasm must therefore be in a condition of tension, and exercise pressure on the internal portions of the cell, which will tend to bring it into the spherical form.

The smaller the mass of protoplasm, supposing it to be homogeneous, the greater will be the pressure exerted by its surface layer on its contents and the greater resistance will it present to deformation of the spherical form. A fluid drop, if suspended in a fluid with which it is immiscible, will present greater rigidity the smaller its dimensions. Almost any degree of rigidity can thus be imparted to an emulsion. In such a case every droplet will present resistance to deformation and every surface will resist penetration or extension. The resistance of the surface in colloidal fluids is still further increased by a property common to all these fluids, namely, the aggregation in the surface layer of a greater concentration of the dissolved substance than is present in the underlying fluid. Protoplasm may therefore be regarded as fluid in character, the form and rigidity which are acquired by most cells being due to physical differentiation in its substance, as when the colloid is a stiff emulsion, or when, as at the surface, there is the formation of a solid condensation layer. In some cells, as for instance, muscle fibres, the cytoplasm on microdissection behaves as a gel. The cytoplasm in many cells is certainly of very low viscosity; when certain sea-urchin eggs are centrifuged, the granules settle in layers within the cell (Fig. 7), and in some species at a rate indicating a viscosity only four times that of water. The physical condition of protoplasm is without doubt subject to considerable and often very sudden changes. When, for instance, a living cell is damaged by a microdissection needle, the fluid homogeneous cytoplasm almost at once sets to a jelly-like semi-solid, in which definite granules soon appear. Bayliss showed that when an amœba was stimulated electrically, while observed by dark-ground illumination, the fine movement of the granules was momentarily checked, also presumably owing to a temporary gelation of the cytoplasm.

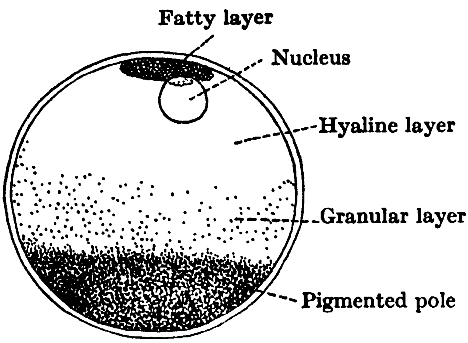


FIG. 7. Stratification in contents of *Arbacia* egg after two minutes' rapid centrifugalisation. (After LYON.)

THE SURFACE LAYER OF CELLS. As mentioned above, colloid solutions have at their surfaces a layer in which the colloid substance is much more concentrated than it is in the body of the solution. If for instance we take a beaker containing egg-white diluted 100 times, and drop a steel magnetised needle on to the surface, it will float in consequence of the resistance of the surface. If the needle be greasy the same thing will occur on water, and in this case the needle will lie N. and S. On the albumin solution, however, the needle will lie in the position in which it has been dropped. The aggregation of the albumin molecules on the surface of the fluid results in the formation

of a solid film which resists any turning of the needle. What applies to the interfacial surface between fluid and air applies also to that between two different fluids. Thus in milk, which is an emulsion of fat droplets in a protein solution, each droplet of fat is surrounded by a layer of concentrated, possibly solid, protein. A similar condensation leading to the formation of a very thin membrane is believed to take place at the surfaces of cells, so that, although the cytoplasm may be liquid, and miscible with water, the contents of the cell are separated from their surroundings by a semi-solid membrane.

Since it is by means of its surface layer that the organism enters into relation with its environment, this layer acquires a prime importance for the life of the cell, and we may therefore consider here at greater length some of the properties of this layer, the *plasma membrane*, as it has been called. This layer is not to be confounded with the cell wall of plant cells, which is generally freely permeable to all kinds of solutions, and so plays no part in regulating the interchanges of the cell with the environment. Every change in the environment can influence the living cell only through its surface layer, through which substances must pass on their way into the cell for assimilation, or out of it for excretion. The retention of an individuality by the cell must be determined by chemical and physical differences between this layer and the surrounding fluid. Since it differs from the rest of the protoplasm in the changes to which it is subject, it must also differ in its chemical composition, apart altogether from the factors which, as we saw above, determine molecular differences between the surface and the interior of any colloidal solution. On this account one must assume the existence of a definite

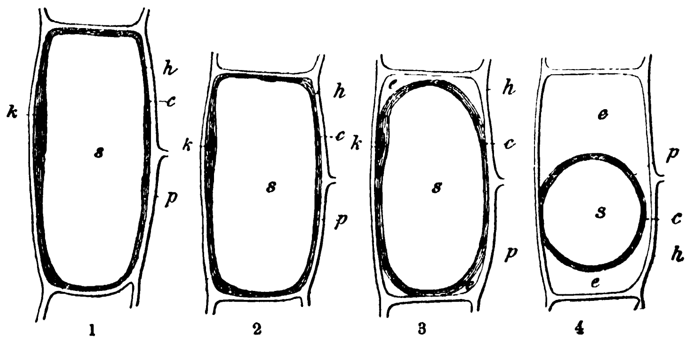


FIG. 8. Vegetable Cells, showing varying degrees of Plasmolysis. (DE VRIES.)

boundary layer of the protoplasm, even where it is impossible to see any differentiation between this layer and the deeper parts under the highest powers of the microscope.

A living cell, which leads its life in a liquid environment, must take up the greater part of its food material in the form of solution; and it is the permeability of the superficial protoplasm which will determine the passage of food substances from the surrounding medium into the body of the cell. The immiscibility of the protoplasm with the surrounding fluid shows that the permeability of the membrane must be a limited one. The qualitative aspect of permeability was first studied in vegetable cells. These present within a rigid cellulose wall a thin layer of protoplasm (the 'primordial utricle'), enclosing a cell sap. If the root hairs of tradescantia be immersed in a 10 per cent. solution of glucose or in a 2 to 3 per cent. solution of salt, a process of *plasmolysis* takes place. The cell sap diminishes in amount by the diffusion of water outwards so that the primordial utricle shrinks (Fig. 8 (4)). On placing the cells in distilled water, water passes into the cell sap until the further expansion of the protoplasmic layer is prevented by the tension of the surrounding cell walls (Fig. 8 (1)). This behaviour can be explained only on the assumption that the protoplasm is impermeable or but slightly permeable, both to sugar and to salt, but is freely permeable to molecules of water, *i.e* it behaves as a semi-permeable membrane.

Similar experiments can be made on red blood corpuscles. These also shrink when immersed in salt solutions with a greater molecular concentration than would correspond to the plasma of the blood from which the corpuscles were derived, whereas if placed in weak salt solutions or in distilled water they swell up and burst, discharging their hæmoglobin in solution into the surrounding fluid (*hæmolysis*). By comparison of

various solutions it is found that the strength of each solution which is just necessary to cause plasmolysis or hæmolysis, as the case may be, is determined entirely by its osmotic pressure, *i.e.* solutions having the same osmotic pressures produce the same results.

The specialised nature of the cell surface is well illustrated by comparing the chemical composition of the cell contents with that of its normal environment. Thus in red blood cells there is an excess of potassium over sodium, whereas in the normal blood serum the reverse is the case. The limited permeability of the plasma membrane also enables the cell to maintain a different reaction, usually more acid than that of its surroundings. Most cells show a cytoplasmic *p*H of about 6·8 to 7·0 when tested by micro-injection of indicator dyes; the nucleus, which again is separated from the cytoplasm by a membrane, usually has a *p*H definitely higher (more alkaline), viz. about 7·6 to 7·8, according to micro-injection experiments by Chambers and Pollack.

The impermeability of the plasma membrane is not explicable on any simple physico-chemical basis, since it does not apply to all dissolved substances. Overton found that, whereas this layer is practically impermeable to salts, sugars and amino-acids, it permits the easy passage of monatomic alcohols, aldehydes, alkaloids, &c. All these substances are more soluble in ether, oil and similar media than they are in water. The passage of dissolved substances through a membrane wetted by the solvent may be supposed to depend on the solubility of these substances in the membrane. A layer of lipoid material one molecule in thickness has been shown to hinder the rate of evaporation of water, but not that of ether.* Overton concluded that the superficial layer of cells must itself partake of a 'lipoid' character, and that cholesterol and lecithin probably enter largely into its composition. He stated that only such aniline dyes as are soluble in a mixture of melted lecithin and cholesterol have the property of penetrating the living cell, and only these dyes, such as methylene blue, and neutral red, can be used for *intra vitam* staining. This does not apply, however, universally. For example, methylene green, thionin and malachite green are not "lipoid-soluble," but penetrate cells readily, whereas cyanosin and Bengal rose are "lipoid-soluble," but will not enter. Moreover, water itself is not sterol-soluble. The kidney cells can take up many dyes which are not 'lipoid-soluble,' such as phenol sulphone-phthalein and indigo-carmine, and the same applies to other gland cells. It may be noted that substances which have the power of dissolving lecithin and cholesterol, such as ether or bile salts, also act as hæmolytic agents, *i.e.* they cause a destruction of the red blood cells by rendering porous the superficial layer which is necessary for their preservation from the solvent effects of the surrounding fluid. An interesting feature of permeability is that weak acids or bases, *e.g.* CO_2, H_2S, HCN, NH_3, penetrate cells readily, whereas strong ones, *e.g.* HCl, NaOH, fail to do so. The semi-permeability of the plasma skin can be altered by changes in the saline concentration or other factors of the surrounding medium. Overton has shown that, whereas a 7 per cent. solution of sucrose produces plasmolysis in living cells, no plasmolysis is observed if they are treated with a solution containing 3 per cent. methyl alcohol *plus* 7 per cent. sucrose. It is as though the superficial layer were able to dissolve a mixture of methyl alcohol and cane sugar, although it had no solvent power on cane sugar in pure watery solutions.

It is possible that in order to serve the nutrient needs of the cells, changes may take place in the permeability of the surface layer under certain conditions. Further, in the case of the red blood cells, if we assume complete and permanent impermeability to cations, it is difficult to explain how the relative concentrations of potassium and sodium in cell and plasma could arise in the first place. When cells are damaged, or dead, and in certain cases also when they are stimulated, a change in permeability occurs, so that cell contents then diffuse freely through into the surrounding medium.

No doubt protein also enters into the composition of the surface membrane and considerably modifies the properties attributable to lipoids. Such a view has, indeed, recently been advanced † (Fig. 9). According to this view the cell surface consists of a solid layer of lipoid only two or three molecules in thickness, but with a film of protein adsorbed upon its surfaces. The lipoid molecules would be arranged perpendicular to the surface, with all their acidic groups pointed in one direction and their basic groups in the opposite direction. Each protein molecule would be sur-

* RIDEAL. *J. Physical Chem.*, 1925, **29**, 1585. LANGMUIR. *Ibid.*, 1927, **31**, 1719.

† DANIELLI and DAVSON. *J. Cell. and Comp. Physiol.*, 1935, **5**, 495.

rounded by a hydrated envelope. Sodium salts would enter such a film, and as water is soluble in sodium salts, water would also enter; but if the sodium were replaced by calcium salts in which water is not soluble, then the membrane would be less permeable to water. Lipoid-soluble substances would easily penetrate the membrane and would also tend to dissolve and weaken it.

Another hypothesis for explaining variations in permeability of cell membranes is that which regards the change as due to a *reversal of colloid phases* * (*v.* p. 73). A membrane of an emulsoid structure may consist of droplets of oil (disperse phase) in a watery medium (continuous phase), and so be permeable to watery fluids, or the phases may be reversed so that we get watery droplets in an oily medium, in which case only oil-soluble materials would pass through. Alteration of phase conditions often depends upon the inorganic ions which are present: thus calcium favours the dispersion of water in oil, and sodium that of oil in water.

An important factor in the process of assimilation by cells must be the relative solubility of the absorbed substances in the cell and its surrounding medium respectively. Thus a mass of protoplasm will tend to absorb from the surrounding medium and to concentrate in itself all those substances which are more soluble in the colloidal system of the protoplasm than in the surrounding fluid; and this process of absorption may be carried to a very large extent, if the dissolved substances meet in the cell with any products of protoplasmic activity, with which they form insoluble compounds so that they are removed from the sphere of action. It is probably by such a process as this that we may account for the accumulation of calcium or silicon in such large quantities in connection with the bodies of various minute organisms. The uptake of dyes by cells is also largely conditioned by the formation, within the cell, of insoluble complexes, as well as by the permeability of the surface layers to the dye.

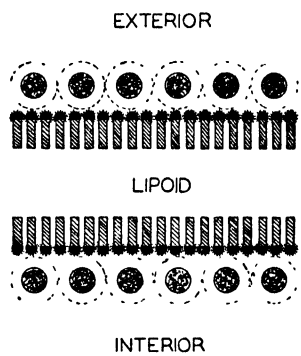


FIG. 9. Schema of molecular conditions at the cell surface. The thin lipoid membrane has its molecules oriented. Outside them are an adsorbed layer of hydrated protein molecules. (DANIELLI and DAVSON.)

Experiments carried out by micro-dissection methods with living cells demonstrate the elastic and semi-solid nature of the peripheral layers of cells, and show that when the surface of a cell is torn or punctured, or even when part of the cell is cut away, the surface film is often at once reformed. It thus appears to be in many cases a condensation layer formed at the surface of separation in accordance with the laws of surface tension. The presence of calcium appears to be essential for the formation of this membrane.

In such animal cells as are connected together by protoplasmic bridges, *e.g.* the cells of the deeper layers of the epidermis, Chambers found that when one of the cells in the mass was damaged, the coagulative process spreads to neighbouring cells.

THE RELATIONS OF THE NUCLEUS TO THE CYTOPLASM. The different staining reactions of nucleus and cytoplasm, and the fact that the nucleus is more opaque to ultra-violet light, suggest a difference in their chemical composition, a suggestion which is confirmed by analysis. In the building up of protoplasm proteins are often combined with other bodies to form complex aggregates. Whereas in the cytoplasm these aggregates consist chiefly of compounds of protein and lecithin, in the nucleus the chief constituents are nucleo-proteins. Material of somewhat similar nature is occasionally found in the cytoplasm, where it forms *chromidial substance*, *e.g.*, the Nissl bodies of nerve cells or the basal filaments of gland cells such as the pancreas.

The readily stainable material of the nucleus, called *chromatin* by histologists, consists mainly of nucleo-protein. It can be distinguished from other stainable cell contents by Feulgen's "nucleal" reaction. This test is carried out by treating sections with warm dilute hydrochloric acid for a few minutes, transferring to fuchsin-sulphurous acid (Schiff's reagent) and washing in dilute sulphurous acid. Chromatin is stained violet owing to the presence of sugars in the animal nucleic acid.

Microscopically, even with dark-ground illumination, the resting nuclear contents

* CLOWES. *Proc. Soc. Exp. Biol. Med.*, 1918, **15**, 107.

are quite homogeneous, except for the presence of nucleoli (Fig. 10). The consistency of the nucleus is found on micro-dissection to be liquid, though perhaps less so than the

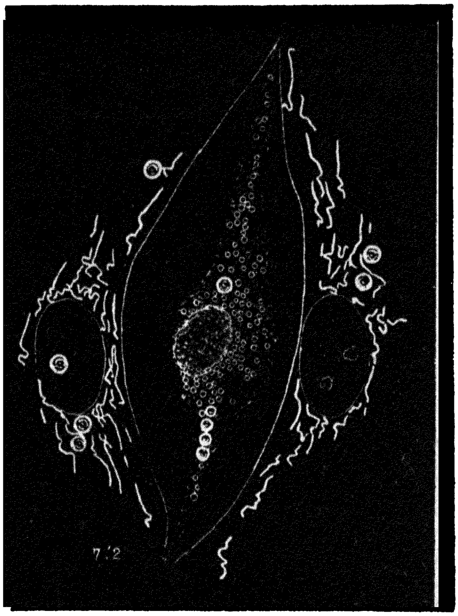


FIG. 10. Three living intestinal epithelial cells in tissue culture, viewed by dark-ground illumination. The nucleus of the middle cell has been punctured by a micro-dissection needle eight minutes previously. The outer boundaries of the two outer cells are invisible, and these cells, with their nuclei and mitochondria appear normal. The cytoplasm of the middle cell has shrunk away from its neighbours and is granular: the mitochondria are also altered. (CHAMBERS and FELL. *Proc. Roy. Soc.*, 1931, **109B**, 380.)

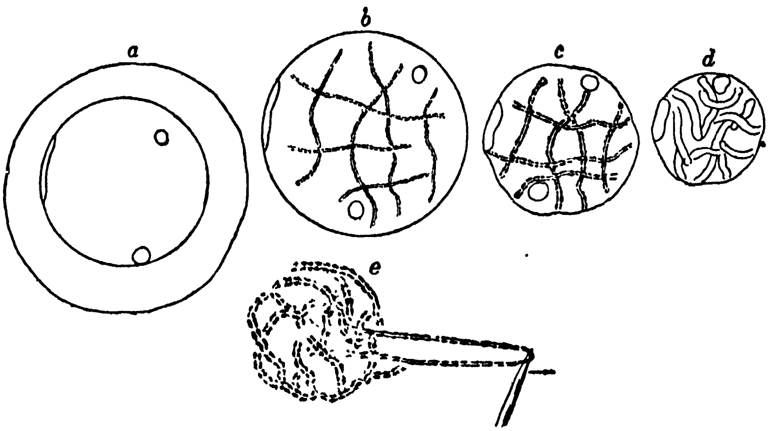


FIG. 11. Living Grasshopper spermatocyte. (*a*) Uninjured; (*b*), (*c*), (*d*) successive stages after injury; (*e*) loop of chromatin filaments stretched with needle. (CHAMBERS.)

cytoplasm; the nucleoli can be freely rolled about within it. When punctured it often rapidly coagulates, and then there may appear, especially in cells about to divide, the characteristic chromatin filaments. These filaments can be drawn out like elastic by

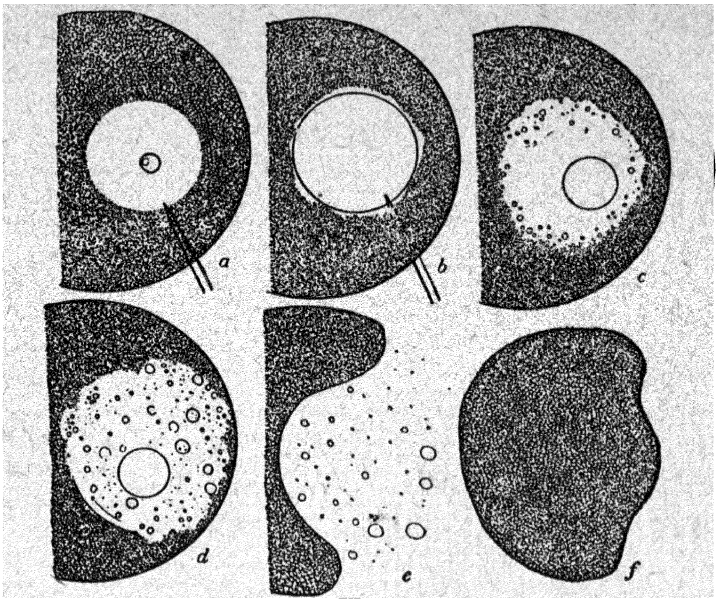


FIG. 12. Disintegration of cytoplasm of starfish egg following puncture of nucleus by micro-dissecting needle. (CHAMBERS.)

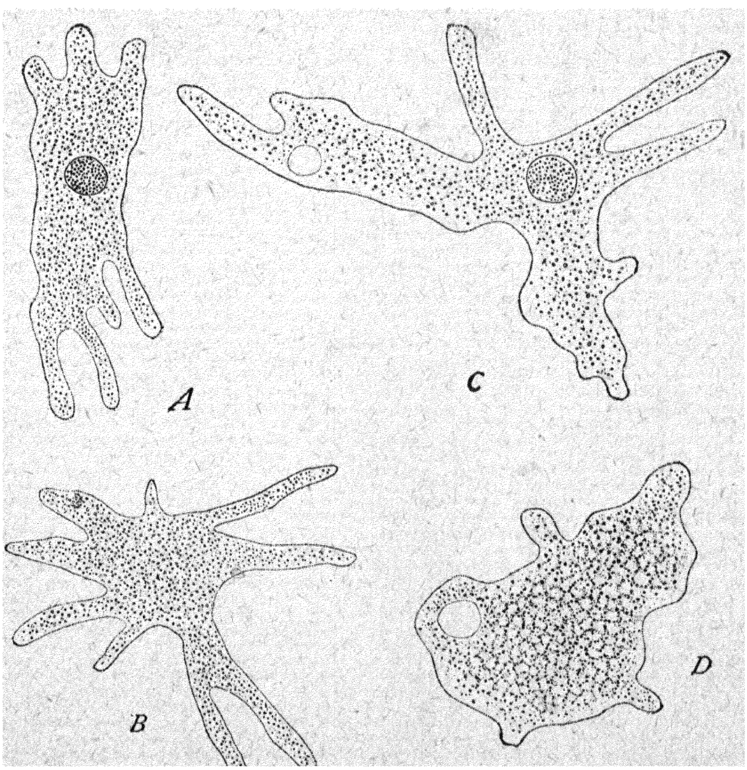


FIG. 13. Nucleated and Non-nucleated Fragments of *Amœba*. (WILSON after HOFER.) *A*, *B*. An *Amœba* divided into nucleated and non-nucleated halves, five minutes after the operation.
C, *D*. The two halves after eight days, each containing a contractile vacuole.

the needle, and retract again when released (Fig. 11). Alternatively puncture of the nucleus may lead to disintegration of both nucleus and cytoplasm (Fig. 12).

By micro-dissection it is possible to divide the larger protozoa into two pieces, one with and one without a nucleus. Hofer, experimenting on the amœba, found that the fragment containing the nucleus quickly regenerated the missing part and pursued a normal existence. On the other hand, the non-nucleated fragments showed no signs of regeneration (Fig. 13). Their movements continued for a short time and then ceased, though the pulsations of the contractile vesicle were but little affected. The power of digestion of food was completely lost. In ciliated mammalian cells, the ciliary movement ceases when the nucleus is destroyed or removed. Fibroblasts in tissue culture often have two nuclei. If the nucleus of a uninucleated cell is punctured, the cell dies, but if one nucleus of a binucleated cell is punctured it continues to live.*

All these facts show that the power of morphological as well as of chemical synthesis depends on the presence of a nucleus.

In the words of Claude Bernard, "the functional phenomena in which there is expenditure of energy have their seat in the protoplasm of the cell (*i.e.* the cytoplasm). The nucleus is an apparatus for organic synthesis, an instrument of production, the germ of the cell."

On this account the nucleus, as we shall learn in the chapter on reproduction is regarded as the special organ of inheritance. The transmission of the paternal qualities from one generation to the next is effected by the entrance simply of the nuclear material of the male cell, the spermatozoon, into the ovum. The important changes which the nucleus undergoes in the process of cell division we shall have to consider more fully later.

Similar conclusions may be drawn from a study of the changes in the nucleus which accompany different phases in the activity of the whole cell. The active growth of cytoplasm, which accompanies the activity of secreting cells, is always associated with changes in the position and in the size of the nucleus.

HISTOLOGICAL DIFFERENTIATION OF CELLS. Even within the limits of a single cell, differentiation of structure can take place by the setting apart of distinct portions of the cell for isolated functions.

The organs which are thus formed in unicellular animals or plants can be divided into two classes, namely (1) temporary organs, which are formed out of a common structural basis and can therefore be replaced at any time by the cytoplasm if destroyed. Examples of such organs are the cilia of unicellular organisms, and the mouth of animals such as vorticella. In contradistinction to these organs are (2) a number of others which must be regarded as permanent. These cannot be formed by differentiation from the cytoplasm of the cell, but are derived by the division of pre-existing organs of the same character, and are therefore transmitted from one generation to another. As examples of such cell organs may perhaps be mentioned the nucleus with its chromosomes, and the mitochondria and plastids.

The possibility of histological differentiation and of the adaptation of structure to definite functions becomes much more pronounced as we pass from the unicellular to the multicellular organisms. The lowest of the Metazoa, such as the sponges, consists of little more than an aggregation or colony of cells. In the Cœlenterata the differentiation is already much more marked. In all these classes of animals the external medium of every cell forming the organism is the sea-water or other medium in which they live, and every cell is therefore exposed to all the possible variations which may occur in the composition of the surrounding medium.

A great step in evolution was accomplished with the formation of the Cœlomata, the class to which all the higher animals belong. In these, by the formation of a body cavity containing fluid, an internal medium is provided for all the working cells of the body. The composition of this internal medium is maintained constant by the activity of the cells in contact with it, and the stress of sudden changes in the chemical composition of the surrounding medium is borne entirely by the outer protective layer of epiblast cells. These are rendered more or less impermeable by the secretion on their surfaces of a cuticular layer, and only such of the constituents of the surrounding medium are allowed to enter the organism as can be utilised by it for building up its living protoplasm. Out of the cœlom is later on formed a circulatory system which, by the circulation of the cœlomic fluid or of blood through the whole body, can procure a still more perfect uniformity in the chemical conditions to which every cell is exposed.

* CHAMBERS and FELL. *Proc. Roy. Soc.*, 1931, **109B**, 381.

One of the main results of the differentiation of function and structure is therefore a gradual setting free of the majority of the cells of the body from the influence of variations in the environment.

The differentiation of the cells, which compose the organs of the body, is determined in the first place by the different conditions to which they are exposed in virtue of their positions in the course of embryonic development. All the higher animals may be considered as built in the form of a tube, the external surface of which is modified for the purpose of defence and for adaptation to changes in the environment. From this layer there are developed not only the protective cuticle, but also the organs of motor reaction, namely, the special senses and the nervous system. The internal surface of the tube is modified for purposes of alimentation. From it are developed all those structures which serve for the digestion of the foodstuffs, for their absorption into the common circulating fluid, for their elaboration after absorption, and their preparation for utilisation by other cells of the body. Between these two surfaces are situated the supporting tissues of the body as well as the muscles. Here also is the cœlom, represented in the higher animals by the pleural, pericardial and peritoneal cavities. The alimentary canal projects for a considerable part of its course into this cœlom, being attached to the body wall only by one side. From the cœlom is also developed the blood vascular system, which maintains a constant circulation of the blood throughout the body. By this differentiation the body becomes divided into a number of organs, each of which is composed of like cells, modified for a common function and bound together by connective tissue, the latter serving also to carry the blood vessels which convey the common medium for the working cells.

The study of the factors which are responsible for the differentiation of the numerous types of tissue cell from the division of the one common cell, the fertilised egg-cell, is a branch of the subject of experimental embryology. It has up to the present not been possible to obtain much beyond a few scattered hints as to what the nature of these factors may be. In the early divisions of some fertilised ova, all the cells appear to be exactly alike. We may believe that to some extent the subsequent course of development of these cells is mechanically determined by their positions and by their relations to one another, but it is certain that this is not the chief factor.

Interesting experiments by Spemann * on embryo newts have shown that at the earliest stages, portions of medullary plate (rudiment of nervous system) and of epidermis can be exchanged, and it is then found that the transplanted portions grow into tissues which are normal for the positions into which they have been transplanted. The position occupied by the tissue is here the decisive factor in its differentiation. But in other experiments it was found that there is a region of the embryo, probably that which later forms the mesoderm, which is not so subjected to its surroundings but which instead determines the formation of definite structures, foreign to their new surroundings. The region is called an "organiser." It seems likely that the directing influence of this region may be due to the presence in its cells of definite chemical substances,† probably a steroid.

During the course of the development of various organs also it would seem that after a certain stage of embryonic development has been reached, local organising influences are set up. For example, the femur of a five-and-a-half-day-chick embryo can be grown *in vitro*, reach more than three times its inital length, and show almost normal form and bone structure ‡ in spite of isolation from its normal surroundings. Similarly, an eye from a three-day-chick embryo can be grown *in vitro*, and shows a remarkable development and differentiation into normal eye tissues.§

Experiments in tissue-culture with embryonic tissues also indicate that chemical factors play a part in causing differentiation.

In the foregoing lines we have spoken of an isolated cell of the body as if it were an independent elementary organism. A better term for such an aggregation of cells as presented by the higher animals is not, however, 'cell colony,' but 'cell state,' since, just as in the state politic, no cell is independent of the activities of the others, but the autonomy of each is merged into the life of the whole. With increasing differentiation there is increasing division of function among the various members of the state, and each becomes less and less fitted for an independent existence or for the discharge of

* Spemann. *Proc. Roy. Soc*, 1927, **102B**, 177.
† Waddington *et al.* *Proc. Roy. Soc.*, 1935, **117B**, 289, 310.
‡ Fell and Robison. *Bioch. J*, 1929, **23**, 767.
§ Strangeways and Fell. *Proc. Roy. Soc.*, 1926, **100B**, 273.

all its vital functions. Thus the life of the organism is essentially composed of and determined by the reciprocal actions of the single elementary parts. It is evident that, if the process of specialisation has gone far enough, a discussion whether each unit has or has not an independent life is beside the mark, since it cannot possibly exist apart from the activities of the other cells. From this aspect the morphological unit is not the cell but the whole organism, and the division of the common cytoplasm into cells is merely a question of size and convenience. There can be no doubt that an important factor in the division of cells is their growth : the cell divides because it grows. With increased mass of living substance it is necessary to provide for increase of surface both of cytoplasm and of nucleus. Whether all the tissues of the higher animals remain in structural continuity by protoplasmic bridges, &c., must be a matter of indifference, since all that is necessary for the interdependent working of the different cells of the body is a functional continuity, and this in the higher animals is affected by the presence of a common circulating fluid and a reactive nervous system connected with all the cells of the body.

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*B. BIOCHEMICAL PRINCIPLES**

CHAPTER III

THE ELEMENTARY CONSTITUENTS OF PROTOPLASM

LIVING things contain no chemical elements peculiar to themselves. Every living organism without exception contains the following elements : carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, chlorine, potassium, sodium, calcium, magnesium, copper and iron. In addition to these thirteen elements others are found in certain organisms, sometimes to a large extent. Of these elements we may mention especially silicon, iodine, fluorine, bromine, aluminium, nickel, cobalt, zinc, lead, silver, cadmium, lithium, strontium and manganese.† Dealing with the first class, which includes those essential to all forms of life, we find that their relative proportions in living organisms have little or no relation to their proportions in the environment of the organisms. Their presence, however, in the latter is a necessary condition of life. In the case of plants growth is limited by the amount of the necessary element (*e.g.* potassium, nitrogen, phosphorus) which is present in smallest quantities in the surrounding medium.

Carbon forms the greater part by weight of the solid constituents of living protoplasm and the proximate constituents of living organisms are practically all carbon compounds, and hence are combustible. In the inorganic world practically all the carbon occurs as carbon dioxide, which is present to the extent of about 0·04 per cent. in the atmosphere, while vast quantities are immobilised in the form of chalk and limestone. In this condition the carbon is practically removed from the life cycle, the whole of the carbon contained in the tissue of living beings being derived from carbon dioxide present in the atmosphere.

The energy for the conversion of carbon dioxide into the oxidisable forms which make up the tissues of plants and animals, is furnished by the sun's rays. The conversion of the radiant energy into the potential chemical energy of the carbon compounds is performed by the chlorophyll corpuscles in the green parts of plants. In these corpuscles, under the influence of the sun's rays, the carbon dioxide of the atmosphere, together with water, is converted into starch, and the oxygen liberated in the process is set free.

$$n\,[6CO_2 + 5H_2O] = (C_6H_{10}O_5)_n + 6nO_2.$$

In this process a large amount of energy is absorbed, which can be set free later by the oxidation of the starch to carbon dioxide. In the oxidation of 1 gramme of starch about 4·5 kilo-calories are evolved, and this represents therefore the measure of the solar energy which must be absorbed by the chlorophyll corpuscle in the process of formation of 1 gramme of starch from carbon dioxide. By this means the world of life is provided with a source of energy. At the expense of the energy of the starch further synthetic processes are carried out and fats and proteins manufactured.

All animals take in carbon, hydrogen, nitrogen, oxygen and sulphur in the form of the carbohydrates, fats and proteins which have been built up in the living plants. In the animal organism these foodstuffs serve as sources of energy.

* Physiology and biochemistry overlap considerably, and it is desirable for the advanced student of physiology to enter more deeply into the biochemical side of the subject than is possible in this volume. For this purpose he may profitably consult :—

BODANSKY. "Introduction to Physiological Chemistry," John Wiley and Chapman and Hall (1934).

THORPE. "Biochemistry for Medical Students" (Churchill, 1938).

in addition to the various references given in the present volume.

† FOX and RAMAGE. *Proc. Roy. Soc.*, 1931, **108B**, 157.

Oxygen is taken up in the free as well as in the combined state. It takes a position apart from the other foodstuffs in that its presence is the essential condition for the utilisation of their potential energy. In the living cells it combines with the oxidisable compounds formed by the agency of the living protoplasm, with the production of carbon dioxide and water and the evolution of energy.

Nitrogen is also derived indirectly from the air. It can be taken up by most plants only in the form of ammonia, nitrites or nitrates. Animals cannot utilise these compounds, and their only source of nitrogen is the protein which has been built up from them by the agency of the plant cell.

Since nitrogen in the free state is useless, the existence of life must depend on the amount of combined nitrogen which is available. There are certain cosmic factors which result in the production of combined nitrogen. Every thunderstorm results in the production of small quantities of ammonium nitrite, which will be washed down with the rain and serve as a source of combined nitrogen to the soil. Many of the higher plants require their nitrogen in the condition of nitrates, and this conversion is effected by micro-organisms. There are a number of bacteria (*bacterium nitrosomonas*) which have the power of converting ammonia into nitrites. Others (*b. nitromonas*) convert nitrites into nitrates. This has been made the basis of a method now largely employed for the treatment of ammoniacal sewage. These different bacteria play an important part in all soils.

Organisms also exist which can bring into combination the free atmospheric nitrogen itself. Thus certain soils have been found to undergo a gradual enriching in nitrogen although no nitrogenous manure has been applied to them. This fixation of nitrogen by soils is effected by a distinct micro-organism, *clostridium pasteurianum*.

In addition to this spontaneous fixation of nitrogen by humus, it has long been known to farmers that the growth of almost any leguminous crop in a soil poor in nitrogen may result in an actual increase of nitrogen in the soil from which the crop is taken. It was found that this was dependent on the presence on the roots of certain small nodules swarming with bacteria. These bacteria, like those of humus, are able to assimilate the free nitrogen of the atmosphere but, unlike them, can effect this assimilation only in a condition of symbosis, *i.e.* living in the growing tissues of a leguminous plant.

Sulphur is found in all soils in the form of sulphates, generally of calcium. In the plant cell it is built up, together with nitrogen, carbon and hydrogen, to form a part of protein molecules. Practically the whole of the sulphur taken in by animals is in the form of proteins. It is oxidised in the animal body which it leaves in the form of sulphates.

Iron, although forming but a minute proportion of the material basis of living organisms (the whole body of man contains only six grammes), is nevertheless indispensable for the maintenance of life. It is necessary for instance in two important functions, viz. the formation of chlorophyll in the green plant and the respiratory process in the higher animals. Iron forms an essential constituent of hæmoglobin, the red colouring matter of the blood, whose office it is to carry oxygen from the lungs to the tissues. The pigment chlorocruorin, present in the plasma of polychæte worms, is an iron compound resembling hæmoglobin. It is probable too that the minute traces of iron in protoplasm exercise an important function in the processes of oxidation which are continually going on. Iron obtained by plants from the soil is built up into highly complex organic compounds, and in this form is taken up by animals. It is probable that the main requirements of the animal for iron, which in the day's diet for man is present to the extent of about 15 mg. in all, may be satisfied entirely at the expense of these organic compounds, but there is little doubt that the animal can, if need be, also utilise the iron salts present in its food. The animal proceeds extremely economically with its supply of iron. Any excess of iron above that needed to supply the iron lost to the body is excreted almost entirely with the fæces in the form of sulphide.

Phosphorus is absorbed by the plant as phosphates. In the cell protoplasm it is built up with fatty acids and other organic radicals to form complex compounds such as lecithin, and nuclein, a combination of phosphoric acid with various nitrogenous bases. Both lecithin and nuclein are essential constituents of living protoplasm. An important function of phosphate in the body is the formation of organic phosphoric esters of various types. The different phosphorus compounds are broken down by processes of dissociation and oxidation, with the production, as a final result, of phosphates which are excreted with the urine or fæces and return to the soil.

Chlorine, potassium, sodium, calcium and *magnesium* are taken up by the plants

in the form of salts; magnesium is one of the elements present in the chlorophyll of plants. They are taken up by animals in the form of salts, and as such are again excreted with the urine.

Little is known about the significance, if any, of the other elements which have been mentioned as frequent constituents of living beings. *Silicon*, which is of very wide distribution, is assimilated as silica, probably in colloidal solution, and is distributed in minute quantities through all plant and animal tissues. It forms a very large percentage of the mineral basis of grasses, but even here it does not seem to be indispensable.

Fluorine is found in the enamel of the teeth and in minute traces in other tissues of the body.*

Bromine, though present in quantity in some seaweeds, appears to play no part in the economy of higher animals.

Iodine is found in large quantities in many seaweeds and sponges. The substance thyroxine, the active principle of the thyroid gland, contains iodine, and is essential for normal life in the higher animals. Iodine therefore is an essential constituent of the higher animals.

Aluminium is found in large quanties in certain lycopods. Whether it is essential to their growth is not known.

Copper in traces is often a valuable constituent of the diet of animals. In the cephalopods and crustacea it appears to take the part of iron in the formation of a blood pigment, hæmocyanin, which plays the same part in the blood of these animals that is played by hæmoglobin in the blood of vertebrates. When oxidised it is of a blue colour, but gives off its oxygen and is reduced to a colourless compound on exposure to a vacuum.

Manganese is widely distributed in traces, and in the lamellibranch *Pinna squamosa* appears to form an essential constituent of a blood pigment, like hæmocyanin in its functions, and called pinnaglobulin. An ordinary mixed diet for man probably contains about 2 mg. each of copper and manganese per day.

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* McClure. *Physiol. Rev.*, 1933, **13**, 277.

CHAPTER IV

THE PROXIMATE CONSTITUENTS

In spite of the enormous variety of the proximate organic constituents of living organisms (and hence of all foodstuffs), most of them are members or derivatives of three classes of compounds. These classes are :—

The Lipides.
The Carbohydrates.
The Proteins.

It is assumed that the student has some acquaintance with the elements of organic chemistry and we shall therefore confine our description here to those aspects which have a direct bearing on the physiology of nutrition and metabolism.

1. THE LIPIDES

The ethereal extract of a tissue contains a variety of fat-soluble substances, many of which are characterised by the fact that they contain fatty acids as integral parts of their molecules. They may be classified into the following groups :—

(1) *Fats.* Neutral esters of glycerol and fatty acids.
(2) *Sterols and Waxes.* Of these the sterols are by far the most important. They are alcohols, the chief sterol of the animal body being cholesterol. Waxes are neutral esters of alcohols, such as cholesterol, cetyl alcohol, etc., with fatty acids.
(3) *Phospholipides* (Phosphatides) are nearly insoluble in acetone, a property which is utilised in their separation from other lipides. Lecithin and kephalin are compounds of an alcohol (glycerol) with two fatty acids, phosphoric acid and an organic base (choline or colamine respectively). Sphingomyelin contains phosphoric acid linked to choline and a base, sphingosine, which in turn is linked to a fatty acid.
(4) *Glycolipides* (Galactosides or Cerebrosides). Compounds of a carbohydrate (galactose), with a fatty acid and an organic base (sphingosine).
(5) *Hydrocarbons.* Very small amounts of substances belonging to the class of lipochrome pigments occur in the tissues. The most important of these is the hydrocarbon carotene, $C_{40}H_{56}$, which is converted into vitamin A in the liver. Another unsaturated hydrocarbon, squalene, $C_{30}H_{50}$, occurs in very large quantities in some shark liver oils.

1. THE FATS

Quantitatively the fats themselves are easily the most important of the lipide groups and they are widely distributed throughout the animal and vegetable kingdoms. In the higher animals they are the main constituents of the fatty or adipose tissue. They also occur as fine particles in the protoplasm of cells and probably also in combination with the other constituents of protoplasm. In contrast to the mammal, certain fish store up their fat

reserve in the liver (*e.g.* cod liver oil), instead of in adipose tissue. Large amounts are also found in certain seeds (cotton seed), and nuts (brazil nut).

COMPOSITION. The fats, the chief representatives of simple lipides, are esters of glycerol and the fatty acids. Glycerol can form esters (glycerides) with one, two or three of its hydroxyl groups. Thus with phosphoric acid the following important compounds are formed :—

$$\begin{array}{ccc} (1) & \quad & (2) \\ CH_2OH & & CH_2OH \\ | & & | \\ CHOH & & CH{-}O{-}PO_3.H_2 \\ | & & | \\ CH_2O{-}PO_3.H_2 & & CH_2OH \\ \alpha \text{ glycerophosphoric acid} & & \beta \text{ glycerophosphoric acid} \end{array}$$

With a fatty acid, such as acetic acid, it forms mono-, di- or triglycerides, *e.g.* :—

$$\begin{array}{ccccc} (3) & \quad & (4) & \quad & (5) \\ CH_2OH & & CH_2OH & & CH_2{-}O{-}OC.CH_3 \\ | & & | & & | \\ CHOH & & CH{-}O{-}OC.CH_3 & & CH{-}O{-}OC.CH_3 \\ | & & | & & | \\ CH_2{-}O.OC.CH_3 & & CH_2{-}O{-}OC.CH_3 & & CH_2{-}O{-}OC.CH_3 \\ \alpha \text{ monacetin} & & \alpha,\beta \text{ diacetin} & & \text{triacetin} \\ \text{(monoglyceride)} & & \text{(diglyceride)} & & \text{(triglyceride)} \end{array}$$

The fats are mainly triglycerides, and natural fat is usually found to consist of various mixed triglycerides ; these are not generally simple esters of the type shown in formula (5), but have different fatty acid radicals combined with the glycerol, and in the animal these fatty acids usually have sixteen or more carbon atoms. The fatty acids which enter into the composition of the triglycerides may be either saturated or unsaturated, belonging to homologous series having the following formulæ :—

Saturated fatty acids : $C_nH_{2n+1}COOH$ *e.g.*

Butyric acid,	C_3H_7COOH
Palmitic acid,	$C_{15}H_{31}COOH$
Stearic acid,	$C_{17}H_{35}COOH$

Unsaturated fatty, acids, e g. :—

Series $C_nH_{2n-1}COOH$	Oleic acid,	$C_{17}H_{33}COOH$	(1 double bond)
,, $C_nH_{2n-3}COOH$	Linoleic acid,	$C_{17}H_{31}COOH$	(2 double bonds)
,, $C_nH_{2n-5}COOH$	Linolenic acid,	$C_{17}H_{29}COOH$	(3 double bonds)
,, $C_nH_{2n-7}COOH$	Arachidonic acid,	$C_{20}H_{31}COOH$	(4 double bonds)

Adipose tissue, so-called, consists almost exclusively of the fats derived from palmitic, stearic and oleic acids, *e.g.* dioleo stearin, oleo-palmito stearin, triolein, etc., *e.g.* :—

$$\begin{array}{l} CH_2O{-}O.CO.(CH_2)_7.CH = CH.(CH_2)_7.CH_3 \; \textit{(Oleate)} \\ | \\ CHO{-}O.CO.(CH_2)_{16}.CH_3 \; \textit{(Stearate)} \\ | \\ CH_2O{-}O.CO.(CH_2)_{14}.CH_3 \; \textit{(Palmitate)} \\ \qquad\qquad \text{Oleostearopalmitin} \end{array}$$

The great differences in the appearance of the fat of different animals are due to the varying proportions in which these different mixed glycerides occur. While triolein is liquid at 0° C., tristearin and tripalmitin are solid at the temperature of the body. According, therefore, to the relative amounts of these three fatty acids present in the mixed glycerides, we may have

a fat which, like mutton suet, is solid at the body temperature, or a fat containing much olein which is still fluid when the body is opened after death, even when it has already cooled. Milk fat is the one animal fat which has peculiar properties in that it contains large proportions of lower fatty acids (butyric acid 5 per cent.), a fact which must be of importance in any theory concerning fat secretion in the mammary gland.

PROPERTIES. The fats are colourless substances devoid of smell. They are insoluble in water, in which they float. They are soluble in warm absolute alcohol, but separate out into crystalline form on cooling, and are easily soluble in ether, petroleum ether, acetone, benzene and chloroform. If they are heated with steam or, more readily, with dilute alkalies, or submitted to the action of certain enzymes, they undergo hydrolysis, and are split into three molecules of fatty acid and one molecule of glycerol, *e.g.*:

$$\underset{\text{(neutral fat—tripalmitin)}}{C_3H_5(OOC.C_{15}H_{31})_3} + 3H_2O = \underset{\text{(palmitic acid)}}{3C_{15}H_{31}.COOH} + \underset{\text{(glycerol)}}{C_3H_5(OH)_3}$$

This process may occur spontaneously when fat is left exposed to the air. Fat is then said to be rancid and has an increased acid value. Most natural fats generally contain a small amount of fatty acid.

Saponification. On boiling a neutral fat for a long time with an aqueous, or for a shorter time with an alcoholic, solution of potassium or sodium hydroxide, the fat, like other esters, undergoes *saponification*, giving the alkaline salt of a fatty acid and glycerol. The former compound is spoken of as a soap. In water the soaps form a sort of pseudo-solution on heating which sets to a solid jelly on cooling. From a dilute watery solution the soap can be thrown down in the solid form by the addition of neutral salts.

Emulsification. If a liquid fat be shaken with water, the droplets of fat become mechanically dispersed through the water, but quickly run together again on ceasing the agitation, so that the mixture separates completely into two layers. If, however, alkali be added to the mixture until it is just alkaline to phenolphthalein—*i.e.* until any free fatty acids have been converted into soluble soaps—a fine creamy emulsion will then result from the shaking, and may remain stable for months. This is attributed to the stabilising effect of the soap upon the dispersed droplets of fat, due to the adsorption of the soap and its accumulation as a pellicle at the interface between water and each fat droplet, thus causing a corresponding reduction of the oil-water surface tension at the interface. The provision of a large surface area by emulsification is a necessity for the action of fat-splitting enzymes. The addition of acid, by precipitating the soaps as insoluble fatty acids, destroys this protective layer and causes the immediate breakdown of the emulsion.

Analysis of Fats. Owing to the difficulty which attends the complete separation of each acid from a mixture of allied fatty acids, such as is obtained by the hydrolysis of a fat, the complete analysis of a sample of a fat is seldom attempted, and in routine work a series of empirical determinations is made which together suffice to give a good deal of information as to the chemical nature of the fatty acids concerned. The following determinations are usual:—

The *melting point.*

The *acid value* of a fat is the amount of potassium hydroxide in milligrammes required to neutralise the free fatty acids in 1 gramme of fat.

The *saponification value* is the amount of potassium hydroxide in milligrammes required to saponify 1 gramme of fat.

The *iodine value* is the amount of iodine in grammes with which 100 grammes of fat will combine.

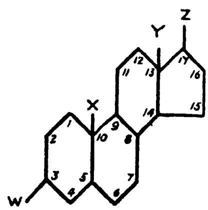
The *Reichert-Wollny value* or *volatile fatty acid value* is the amount of N/10 potassium hydroxide in c.c. required to neutralise the volatile fatty acids in 5 grammes of hydrolysed fat.

The acid value gives an indication of the acidity or rancidity of the fat. The saponification value gives some idea of the mean molecular weight of the total fatty acids. The Reichert-Wollny value indicates the proportion of these which are volatile in steam (*i.e.* are of low molecular weight); and the iodine value represents the mean degree of unsaturation of the acids of the fat.

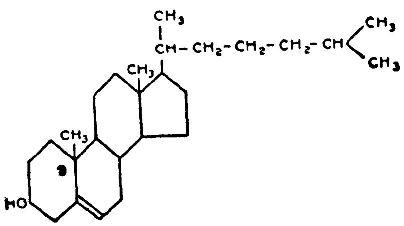
2. THE STEROLS

A certain number of substances occur in the body derived from a formation of esters of the fatty acids with sterols such as cholesterol. The fatty secretion of the sebaceous glands in man and the higher animals, which furnishes the natural oil of hair, wool (lanolin) and feathers, consists of cholesteryl esters with small traces of glycerides. The sterols also occur in the cells in the free state.

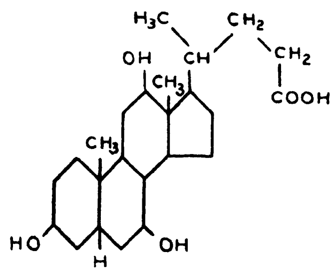
The sterols are mostly complex monatomic alcohols which are widely distributed throughout all the cells and fluids of the body. They contain the characteristic "*cholane*" ring:—



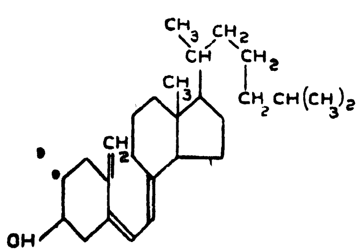
The most widespread member is *cholesterol,* an unsaturated secondary alcohol, $C_{27}H_{45}OH$, which is possibly the precursor in the body of the numerous derivatives. Its corresponding saturated compound, *coprosterol,*



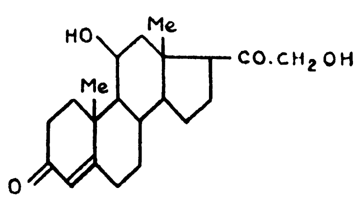
Cholesterol.



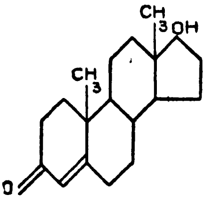
Cholic acid



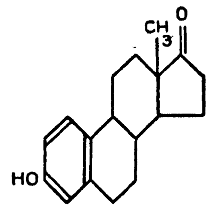
Vitamin D_3.



Corticosterone.



Testosterone



Œstrone

is found in fæces, while the closely related *ergosterol*, $C_{28}H_{43}OH$, yields vitamin D on irradiation. All the sterols are very stable, and resist the action of enzymes and most bacteria.

The sterols are now recognised as a very important class of biological compounds. How closely related chemically are some substances of widely different physiological properties may be seen by the formulæ given above for cholesterol, a bile acid, a vitamin, a suprarenal hormone (corticosterone), a male sex hormone and a female sex hormone (œstrone).

Cholesterol is an important constituent of all cells and it, or its esters, forms part of the framework of the living cells, in the interstices or at the surfaces of which the numerous biochemical reactions associated with their life processes proceed.

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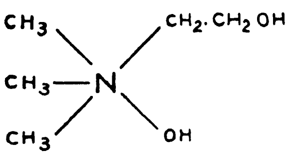
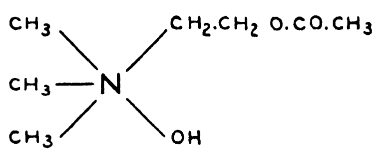
3. PHOSPHOLIPIDES

The chief phospholipide is *lecithin*, which contains glycerol, two molecules of fatty acid, phosphoric acid, and the base choline, a substance which has recently acquired considerable importance owing to the potent physiological

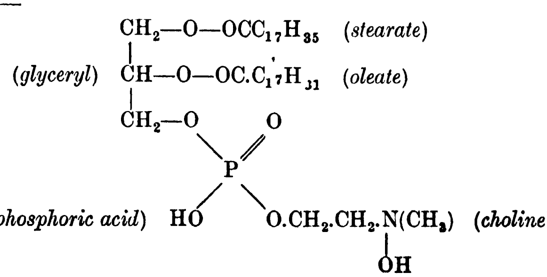


Choline



Acetyl choline

action of its derivative, acetyl choline. Various lecithins, differing only in the nature of the fatty acids contained in them, have been prepared from different tissues. In general, their fatty acids are much more highly unsaturated than those of the food or the reserve fats of the animal—a fact which might suggest that the desaturation of fatty acids is an important preliminary to their metabolism. One of the various lecithins is represented by the following formula in which the two fatty acids are stearic acid and oleic acid :—



Other more unsaturated acids, linoleic, linolenic or arachidonic acids, are however usually present.

Lecithin is a substance very unstable to oxygen, owing to the highly unsaturated acids present in it, and for this reason extreme precautions must be taken in its preparation from tissues, if it is desired to avoid contamination with complex oxidation products. Much of the work on the physiological effect of the phospholipides is probably vitiated by the presence of these impurities. On the other hand, the universal distribution of lecithin signifies an important function in cellular activity. It undoubtedly plays an important part in the transport and metabolism of fats, related on the one hand to the fact that it is capable of colloidal solution in water, facilitating its free transport and, on the other, to the possibility that desaturation is involved in its production from neutral fats. Some recent work, however, suggests that the highly unsaturated acids of lecithin may be derived direct from those present in the ingested food. The importance of the constituent base choline in the control of the amount of fat in the liver is discussed later.

It is not improbable that the phosphoric acid of the phospholipides is concerned in the synthesis of the phosphorus-containing nucleoproteins of the cell, and perhaps also in the normal processes of carbohydrate metabolism. Apart altogether from the chemical significance of its constituents, it is certain that lecithin and the allied substances, by virtue of their special physico-chemical condition in solution, play an important part in determining the peculiar permeabilities, osmotic relations and surface conditions of the cell.

Kephalin has a structure identical with that of lecithin, save that the choline of the latter is replaced by amino ethyl alcohol (colamine), $CH_2NH_2CH_2OH$, linked to the phosphoric acid through its alcoholic hydroxyl group.

4. THE GLYCOLIPIDES

The glycolipides are found chiefly in nervous tissue, and are generally prepared from the acetone extract of brain. They do not contain phosphoric acid. The organic base present is a complex substance called sphingosine, present with a higher fatty acid and galactose. They are relatively more stable to oxygen than is lecithin.

The characterisation of individual conjugated lipides is a matter of much difficulty owing to their instability, to the similarity of their general properties and to their eccentric solubilities.

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2. THE CARBOHYDRATES

The Carbohydrates are a group of bodies of wide distribution and great importance in both the vegetable and animal kingdoms. In plants the first product of assimilation of carbon is a carbohydrate, and in animals these substances form one of the most important sources of energy. They consist of the elements carbon, hydrogen and oxygen, the two last-named being almost invariably in the proportions necessary to form water. Their general

formula might be expressed $C_nH_{2n}O_n$. Many of the carbohydrates which are of importance in the animal economy contain six carbon atoms or a multiple of this number. Analogous substances, however, can be prepared containing less or more than this number of carbon atoms. A series of compounds exist which contain in their molecule 2, 3, 4, 5, 6, 7, 8, 9 carbon atoms, and are termed dioses, trioses, tetroses, pentoses, hexoses, heptoses, and so on; the termination 'ose' with the Greek numeral prefixed, indicating the number of carbon atoms, gives them a distinct designation. They are all either ketones or aldehydes of polyatomic alcohols, and known as *ketoses* and *aldoses* respectively.

THE TRIOSES

Two important trioses are glyceric aldehyde (an aldose) and dihydroxy-acetone (a ketose). Both can be obtained by oxidation of glycerol. Their relation to glycerol and to other physiologically important 3-carbon substances are shown below :—

CH_2OH	CH_2OH	CH_2OH	CH_2OH
|	|	|	|
$CHOH$	$CHOH$	$C = O$	$CHOH$
|	|	|	|
CH_2OH	CHO	CH_2OH	$COOH$
glycerol	glyceric aldehyde	dihydroxy-acetone	glyceric acid
CH_3	CH_3	CH_3	CH_2
|	|	|	||
$CHOH$	$C = O$	$C = O$	$C.O.PO_3H_2$
|	|	|	|
$COOH$	CHO	$COOH$	$COOH$
lactic acid	methyl glyoxal	pyruvic acid	phospho-pyruvic acid

Other important related compounds are —

$COOH$	$COOH$
|	|
$CHOH$	$CHO.PO_3H_2$
|	|
$CH_2O.PO_3H_2$	CH_2OH
3-phosphoglyceric acid	2-phosphoglyceric acid

THE PENTOSES.

These bodies occur largely in plants in the form of complex polysaccharides, the Pentosans, which give pentoses on hydrolysis with acids. Three forms of pentose have been found in the animal body, namely, i-arabinose, which has been isolated from the urine in cases of pentosuria, and γ-ribose and desoxy-ribose* which occur built up into the nucleic acid molecule of the pancreas and other organs. The pentoses can apparently be utilised by herbivora as foodstuffs. We know nothing as to the part they play in the animal body or as to the causation of the rare condition of pentosuria.

THE HEXOSES

It is convenient to call those compounds containing six carbon atoms the sugars, because it is to this group that the natural sugars belong.

Stereoisomerism in the Sugars. It will be noticed that of the six carbon atoms contained in the hexose molecule, *e.g.* the aldose $CHO.(CHOH)_4.CH_2OH$, four are *asymmetric*, *i.e.* their four combining affinities are combined with

* LEVENE and TIPSON. *J. Biol. Chem.*, 1932. **94**, 809.

groups of different kinds, viz. two carbon chains, one H atom, and one OH group

$$\begin{array}{c} | \\ -C- \\ | \\ H-C-OH \\ | \\ -C- \\ | \end{array}$$

They must therefore present many stereoisomeric forms. If n represent the number of asymmetric carbon atoms in a compound, the possible number of stereoisomers is 2^n. Thus an aldohexose with four asymmetric carbon atoms $(CHOH)_4$ must present 2^4 isomers, *i.e.* sixteen isomeric compounds, so that there must be sixteen sugars all possessing the formula $CH_2OH(CHOH)_4CHO$, in addition to the inactive sugars obtained by a mixture of two oppositely active members of this group. Only a small number of the sugars are of any physiological importance. These include the aldoses glucose, mannose and galactose, and the ketose fructose or lævulose.

Since these sugars can be divided into the optically active and the inactive varieties, an obvious mode of designation would be to represent them as d-, l-, and i- varieties respectively, *i.e.* dextro-rotatory, lævo-rotatory, and inactive. On Fischer's suggestion, however, this mode of nomenclature has been altered in favour of representing by the letter prefixed, not the optical qualities of the substance in question, but its relation to glucose. Thus d-fructose means that fructose is the ketose corresponding to the dextro-rotatory glucose, d-fructose itself being lævo-rotatory, though its active asymmetric C atoms are identically arranged with those in glucose. With this definition one may say that it is only the d-hexoses of a particular form which are assimilable, and therefore of physiological importance. The small differences in the configuration of the four d-sugars can be readily seen if their graphic formulæ, which for simplicity are shown as straight chains, be compared :

CHO	CHO	CH_2OH	CHO
H.C.OH	HO.C.H	CO	H.C.OH
HO.C.H	HO.C.H	HO.C.H	HO.C.H
H.C.OH	H.C.OH	H.C.OH	HO.C.H
H.C.OH	H.C.GH	H.C.OH	H.C.OH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
d-glucose	d-mannose	d-fructose	d-galactose

Only four hexoses out of the large number which have been synthesised are assimilable by the animal body. These are mannose, glucose, galactose and fructose. They may be synthesised in several ways. The most interesting, because it probably represents the mechanism of synthesis of hexoses in plants, is the formation from formaldehyde. In alkaline solutions, formaldehyde polymerises with the formation of a mixture of hexoses known as acrose. From this mixture α-acrose can be isolated by the formation of its osazone and the reconversion of this osazone into sugar. It is found to be identical with i-fructose.

d-GLUCOSE is the chief constituent of the sugar of fruits. It occurs in the body as the end product of the digestion of starch. It forms white crystals which melt at 100° C. and lose the one molecule of water of crystal-

lisation at 110° C. It is easily soluble in water, and the solution shows mutarotation. Its final specific rotatory power at 20° C. is $[\alpha]_D = +52{\cdot}74°$.

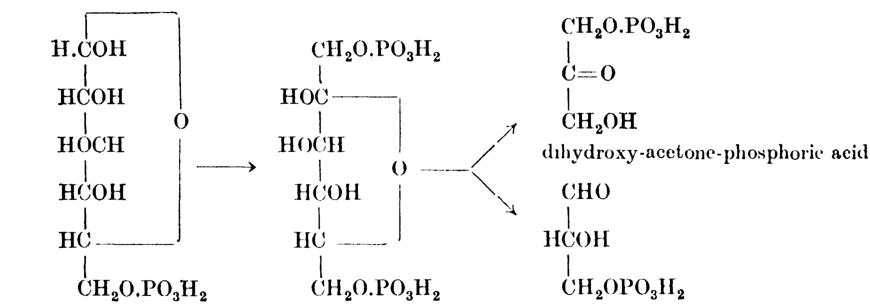
d-FRUCTOSE or LÆVULOSE occurs mixed with glucose in honey and in fruit sugar. It is also, with glucose, formed by the digestion or inversion of cane sugar. It is crystallisable with difficulty. Its watery solution is lævo-rotatory, and reduces Fehling's solution somewhat less strongly than glucose, its reducing power being 92, if we take that of glucose as 100. It ferments readily with yeast; with phenyl hydrazine it gives the same osazone as is formed from glucose.

d-GALACTOSE is formed by the hydrolysis of milk sugar or the galactosides of the brain with dilute mineral acids, and by the hydrolysis of certain vegetable gums. It is much less soluble in water than glucose. It is dextro-rotatory and shows marked mutarotation. With ordinary yeast it ferments, but extremely slowly. Its reducing power is somewhat less than that of glucose.

MANNOSE. Mannose, though an assimilable sugar, is of such rare occurrence in our foodstuffs that it plays practically no part in animal physiology. It is dextro-rotatory, reduces Fehling's solution, ferments easily with ordinary yeast, and gives an osazone which is identical with that derived from glucose.

DERIVATIVES OF THE HEXOSES

The phosphoric esters of the hexoses play an important part in the intermediary metabolism of carbohydrates, since they break down into triose phosphates (*v.* p. 158):—



glucose 6-monophosphoric acid ("*Robison Ester*") — fructose 1, 6, di-phosphoric acid ("*Harden-Young Ester*") — glyceric aldehyde-phosphoric acid

The so-called "Embden ester" is a mixture of glucose-6- and fructose-6-phosphates. The "Cori ester," which is unstable and, in presence of Mg, readily converted into the Robison ester, is a glucose 1-phosphate.

Asymmetric syntheses of sugars from phosphorylated derivatives have been brought about by the use of enzymes from muscle and yeast; these bring about an aldol condensation between dihydroxyacetone phosphate and an aldehyde.* Thus with *d*-glyceraldehyde, fructose 1-phosphate results. It is probably by some such means that sugars are interconverted in the body.

Two other derivatives of glucose are of physiological importance, namely, glucosamine and glucuronic acid.

GLUCOSAMINE has the structural formula:—

$$CH_2OH.(CHOH)_3.CHNH_2.CHO$$

* LOHMANN. *Angew. Chem.*, 1936, **49**, 327.

It is obtained from chitin, which forms the exoskeleton of large numbers of the invertebrata, by boiling this with concentrated hydrochloric acid. It is obtained as a decomposition product of the glucoproteins, such as the mucins. In solution it is dextrorotatory, reduces Fehling's solution, and gives an osazone resembling that derived from glucose.

GLUCURONIC ACID may be regarded as one of the first results of oxidation of the glucose molecule. The group which has undergone oxidation is not the readily oxidisable CHO group, but the CH_2OH group at the other end of the molecule. The formula of this acid is therefore :

$$COOH.(CHOH)_4.CHO$$

It may be produced by the oxidation of glucose by means of hydrogen peroxide. In the free state it does not occur in the animal body. It is constantly found in the urine after administration of certain drugs such as phenol, camphor or chloral, and then occurs as a conjugated acid with these substances. These glucuronates are lævo-rotatory, though the free acid is dextro-rotatory. In the free state it reduces Fehling's solution and gives an osazone which is not sufficiently characteristic to distinguish from glucosazone. It does not undergo fermentation with yeast.

THE FORMATION OF GLUCOSIDES

The formulæ already given for the hexoses suffice to explain the majority of the reactions of the sugars ; but a study of the behaviour of the reducing sugars in solution indicates that they possess a structure more labile than is suggested by the conventional static formula. A sample of glucose which has been recrystallised from water exhibits when dissolved in water a progressive decrease in rotatory power over a period of about twenty-four hours, during which the specific rotation falls from about 100° to a constant value of 52·5°. On the other hand, a specimen of glucose recrystallised from pyridine gives, upon solution in water, an initial specific rotation of about 20°, rising in the same period to 52·5°. This phenomenon receives the name of mutarotation, and is understood to indicate that in solution glucose exists as an equilibrium mixture of two optical isomers which are designated α and β glucose respectively. α Glucose is less soluble in water than the β form, and so is the isomer usually handled as solid glucose. Pure α glucose is obtained by crystallisation from 80 per cent. acetic acid at 20° C. On the other hand, β glucose is obtained when the sugar is crystallised above 98° C. from water, 90 per cent. acetic acid, or from pyridine.

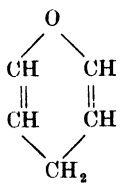
These considerations receive confirmation from the preparation of two monomethyl derivatives of glucose. These derivatives—the methyl glucosides—are the methyl ethers of the two forms of glucose, which have been stabilised by the introduction of the methyl group. Upon hydrolysis they yield glucoses which exhibit a downward and an upward mutarotation respectively.

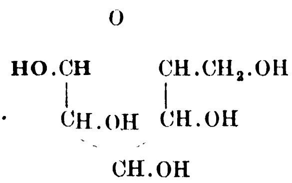
The structural form of these isomers was cleared up by methylation of the sugars whereby all the hydroxy groups may be converted into methyl esters, which renders it possible to decide to which of the carbon atoms of the sugar the hydroxyl groups are attached. In the case of glucose a pentamethyl glucose is obtained in which the methyl groups appear to be attached to the carbon atoms 1, 2, 3, 4, 6 (numbering from the aldehyde carbon). This means that every carbon atom of glucose except the fifth carries a hydroxyl group, and can only be explained by the existence in the molecule of a lactone ring.

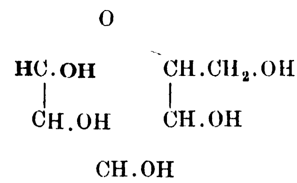
$$\begin{array}{l} \qquad\qquad\qquad\quad O \\ CH_2OH.CH.CHOH.CHOH.CHOH.CHOH \\ \;\;6 \qquad\;\; 5 \qquad 4 \qquad\; 3 \qquad\;\; 2 \qquad\;\; 1 \end{array}$$

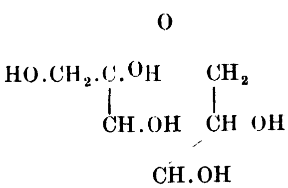
It will be observed that by this modification an asymmetric carbon atom has been created in position 1, accounting at once for the two labile (α and β) forms of the sugar.

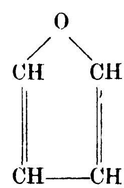
It has also been shown in other ways that in solution under ordinary conditions an oxygen is linked as a 1 : 5 (amylene oxide) linkage on to the carbon chain, giving compounds which may be considered as derived from a six atom heterocyclic ring called Pyran. According to the nomenclature proposed by Haworth these sugars are called pyranoses, *e.g.* glucose could exist as α or β gluco-pyranose ; a 1 : 4 or γ lactone (butylene oxide) linkage can also exist, being a very reactive form called γ glucose (or gluco-furanose, owing to its relation to the 5-atom ring called Furan) of which again there are α and β forms. It is similar with other sugars.

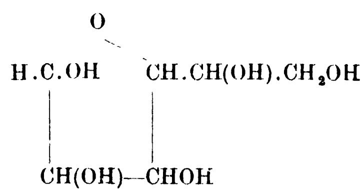

Pyran


β-glucose
(=β-gluco-pyranose)


α-glucose
(= α-gluco-pyranose)


Fructose
(Fructo-pyranose)


Furan


γ glucose (α-form)
(= α-gluco-furanose)

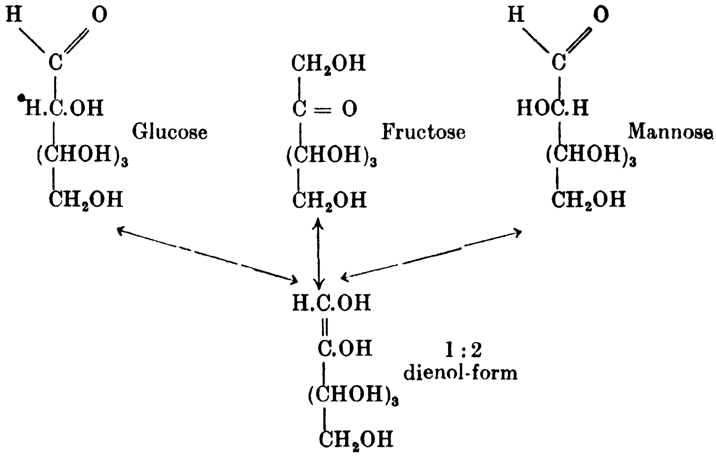
A solution of a hexose must therefore be regarded as an equilibrium mixture of the α and the β forms. The addition of a trace of alkali to a fresh solution of glucose immensely accelerates the rate of mutarotation, indicating an increased lability of the sugar molecule in alkaline solution.

A large number of glucosides occur as plant products—amygdalin, salicin, phloridzin, indican, saponin—and appear to be β glucosides, *i.e.* are ethers formed from β glucose and an alcohol. The disaccharides are also glucosides : thus maltose is glucose α glucoside, whilst lactose is probably glucose β galactoside.

THE ACTION OF ALKALI ON THE HEXOSES. We have seen that a trace of alkali greatly accelerates the mutarotation of the hexoses, indicating an increased lability of the carbon atom at position 1. In more strongly alkaline solutions further changes occur. In the case of glucose a progressive fall in rotatory power reaching a lævo-rotation will be observed. Examination of the solution will then show the presence not only of glucose but also of fructose and mannose.* Since the differences between these three hexoses are confined to the carbon atoms 1 and 2, it will be seen that their ready interconversion in alkaline solution may be

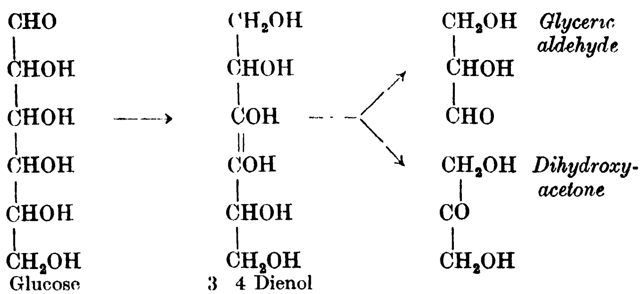
* NEF. *Annalen der Chemie,* 1910, 376, 1 ; 1914, 403, 204.

explained as occurring through the intermediate production of a 1 : 2 dienol-form.



It is of interest that these three hexoses, which are most nearly related *in vitro*, are also the most readily assimilated by the animal. The difference is that, whereas alkali effects an indiscriminate interconversion of glucose, fructose and mannose, the living cell shows directional activity in turning them all into glucose. The anomalous position of galactose as an assimilable sugar is not capable of so facile an explanation on structural lines.

In somewhat stronger alkaline solutions and in absence of oxygen, the 3 : 4 dienol-form results, and this readily breaks up into two important 3-carbon derivatives, glyceric aldehyde and dihydroxyacetone :—



In strongly alkaline solutions in presence of oxygen still more extensive changes occur. A large number of different acids containing varying numbers of carbon atoms, such as oxalic, lactic, tartaric and saccharic acids, and complex resinous substances are formed. There is so much in common between the alkaline and the biochemical degradation of carbohydrate that one could expect that the same broad lines are followed in each, though in the living cell the notable distinction is the production of a selected few of the substances appearing in alkaline solution *in vitro*.

THE DISACCHARIDES

The disaccharides are formed by the union of two molecules of monosaccharides with the elimination of one molecule of water, and can be regarded as glucosides, galactosides, &c. On hydrolysis, *e.g.* on heating with acids, they

take up one molecule of water and are split up into the corresponding monosaccharides. Thus cane sugar gives glucose and fructose, maltose gives two molecules of glucose, while lactose gives glucose and galactose.

CANE SUGAR, or sucrose, is widely distributed throughout the vegetable kingdom, and forms an important article of diet. It has no reducing power, is strongly dextro-rotatory and has a specific rotatory power of + 66·5°. On hydrolysis it is converted into glucose and fructose. Owing to the fact that fructose rotates polarised light more strongly to the left than glucose does to the right, the mixture of the two monosaccharides so obtained is lævo-rotatory. On this account the change to the mixture of monosaccharides is known as *inversion*. With yeast, cane sugar first undergoes inversion by a special enzyme present in the yeast (*invertase*), and the mixture of fructose and glucose is then fermented. Invertase is also present in the intestinal juice.

MALTOSE is formed during the hydrolysis of starch by acids or by the enzyme amylase, and is the chief sugar in germinating barley or malt. It is strongly dextro-rotatory, ferments easily with yeast, and reduces Fehling's solution; its reducing power is about 70 per cent. of that of glucose. With phenyl hydrazine it gives phenyl maltosazone, which forms characteristic yellow crystals with a melting-point of 206° C. The enzyme *maltase*, of the intestinal juice, converts it to glucose.

LACTOSE is a constituent of milk. It is much less soluble in water and much less sweet than are the two other disaccharides. It is dextro-rotatory, shows mutarotation and is not fermented by ordinary yeast. Lactose can be split by the agency of an enzyme, *lactase*, which occurs in the animal body and in certain moulds, into glucose and galactose. Lactose reduces Fehling's solution and gives an osazone, which is easily soluble in hot water.

THE POLYSACCHARIDES $(C_6H_{10}O_5)n$

There are complex substances of high molecular weight formed by combination of monosaccharide molecules, with elimination of water. In the animal body the sole significance of polysaccharides lies in their value as foodstuffs. In plants, anhydrides both of hexoses (hexosans $(C_6H_{10}O_5)_n$) and pentoses (pentosans $(C_5H_8O_4)_n$ occur in bewildering variety. Unlike the other carbohydrates, the higher polysaccharides are not destroyed by hot strong alkaline solutions. The chemical constitution of the polysaccharides is a difficult problem because of their complexity and their colloidal behaviour. The methylation methods of Irvine have given us a deeper insight into the manner of combination of the hexoses in a polysaccharide molecule. It appears certain that glycogen and starch are both composed of α-glucopyranose molecules, linked up as maltose units.

The enzymic synthesis of polysaccharides resembling starch and glycogen has been effected, the starting-point being glucose 1-phosphate.*

STARCH is present in large quantities in nearly all vegetable foods, and is an important constituent of the cereals, as well as of tubers, such as the potato. In the plant cells it occurs as concentrically striated grains, insoluble in water. In hot water the grains swell up and burst, forming a thick paste, which sets to a jelly on cooling. This colloidal solution, as well as the original starch grains, gives an intense blue colour on the addition of iodine. On treating with cold dilute acid, it is converted into a soluble modification, the so-called soluble starch or *amylodextrin*, which also gives a blue colour

* CORI *et al.* *Science* 1939 **89**, 464. HANES. *Proc. R. S.*, 1940, **128 B**, 421; **129 B**, 174.

with iodine. This modification is likewise produced as the first stage of the action of the enzyme *amylase* upon starch. On boiling with dilute acids, starch is converted first into a mixture of dextrins, then into maltose, and finally into glucose. On acting upon starch with amylase, such as the diastase of germinating barley, or with the amylase occurring in saliva or pancreatic juice, it undergoes hydrolysis, the final result of the action being a mixture of four parts of maltose to one part of stable dextrin. In the intermediate stages in this reaction the first product is soluble starch or *amylodextrin.* This breaks up into a reducing sugar and another dextrin, *erythrodextrin,* which gives a red colour with iodine ; and this dextrin, on further hydrolysis, yields reducing sugar and *achroodextrin,* which is not coloured by the addition of iodine. Thus there is a series of successive hydrolytic decompositions of the molecule, each resulting in the splitting off of a molecule of sugar and the production of a lower dextrin. The starch grain is believed to consist of two substances, *amylose* and *amylopectin,* in the ratio 2 : 1, of molecular weights about 60,000 and 300,000 respectively. Each consists of about 24 glucose units, the high molecular weights being due to aggregation. Amylopectin is closely associated with phosphoric acid, with which it may indeed be in combination. On acid hydrolysis both yield glucose ; on hydrolysis with barley diastase, amylose yields maltose, but amylopectin is only partially depolymerised, though oat diastase can split it also finally into maltose.

The Dextrins are ill-defined bodies which are difficult to separate. They are amorphous white powders, easily soluble in water, forming solutions which, when concentrated, are thick and adhesive. They are insoluble in alcohol and ether. With cupric hydrate and caustic alkali they form blue solutions, which reduce slightly on boiling. They are not precipitated by saturation with ammonium sulphate. On boiling with dilute acids, they are converted entirely into glucose.

Inulin, which somewhat resembles starch, occurs in dahlia tubers. It is easily hydrolysed by weak acids into d-fructose.

Glycogen is a polysaccharide which closely resembles starch in structure and properties. It is found in the liver, muscles and other tissues of the body, in all fœtal tissues, and in yeast. It is a white powder forming an opalescent solution in water, and is precipitated from its solution on the addition of alcohol to 60 per cent., by strong acetic acid, by basic lead acetate, or by saturation with solid ammonium sulphate. On boiling with acids, it is entirely converted into glucose. It is affected by amylase in the same way as starch, giving first dextrins and finally a mixture of maltose and dextrin. With iodine it gives a mahogany-red colour which, like the blue colour produced in starch, is destroyed by boiling, to return again on cooling. There are two forms of glycogen, one with 12 and one with 18 glucose units.

The Celluloses. Cellulose is a colourless, insoluble material, or mixture of materials, which forms the cell walls of plants, and is therefore a constituent of most of our vegetable foods. It is insoluble in water or dilute acids or alkalies, but soluble in ammoniacal cupric oxide solution. On boiling with strong acids, it gradually undergoes hydrolysis and yields glucose. In herbivorous animals cellulose can be digested and forms an important constituent of their food. The solution of the cellulose in this case is carried out by the agency of micro-organisms which swarm in the paunch of ruminants and in the cæcum of other herbivora. In some cases the effective agent is a *cytase* present in the vegetable cells themselves. In man cellulose undergoes practically no change in digestion, and serves

merely by its bulk to promote peristalsis and the normal evacuation of the bowels.

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3. THE PROTEINS

The Proteins form the most important constituent of living protoplasm, and must always be present in the food. Owing to their complexity of structure it is not easy to give a simple definition in chemical terms of what we mean by the term 'protein.'

Elementary Composition. All proteins contain oxygen, hydrogen, nitrogen, carbon and, usually, sulphur. The range of the proportion of these elements in the various proteins may be represented as follows:

C	50·6–54·5	per cent.
H	6·5– 7·3	,, ,,
N	15·0–17·6	,, ,,
S	0·3– 2·2	,, ,,
O	21·5–23·5	,, ,,

Many contain a small trace of phosphorus varying from 0·4 to 0·8 per cent., but it is doubtful whether this forms an integral part of the protein molecule.

Physical Characters. The proteins are tasteless colloids, many of which can be crystallised. Most of them are soluble either in water, weak salt solutions, or in dulute acids or alkalies. They are extremely liable to undergo the change called *denaturation,* the nature of which is obscure, as a result of many kinds of chemical or physical treatment. They then tend to become insoluble at the iso-electric point, and this change is generally thought to be irreversible. This denaturation may result from shaking, change of temperature, alteration of reaction, or addition of neutral salts. *Coagulation* by heat forms a distinguishing feature of a number of members of this class, which are therefore spoken of as 'coagulable proteins.' For instance, white of egg is a solution of different proteins. If, however, the solution be heated to about 80° C. a precipitate of coagulated protein is formed. If a strong solution be boiled the whole fluid sets to a solid white mass or *gel.* This change is irreversible, *i.e.* it is not possible by lowering the temperature to bring the white of egg again into solution, and many properties of the protein have been changed in the act of coagulation. A few proteins, *e.g.* gelatin, form gels at lower temperatures, but melt reversibly on warming.

THE STRUCTURE OF THE PROTEIN MOLECULE

We can arrive at some idea of the manner in which the protein molecule is built up by breaking it down by hydrolysis.

By prolonged acid hydrolysis we obtain a complex mixture of substances, nearly all of which are α-amino-acids, and must be regarded as the proximate constituents of the protein molecule.

A similar hydrolytic change may be effected by the use of digestive enzymes. Thus we may use pepsin, trypsin, erepsin, and so on. These

enzymes are all milder in their action than the strong acids. Pepsin for instance effects only a partial decomposition of the protein molecule. Its action results in the formation of substances which still present all the protein reactions and are classified as hydrated proteins or as proteoses and peptones. Trypsin carries the protein a stage further and gives a mixture of polypeptides and amino-acids. Erepsin breaks the polypeptides down to amino-acids.

THE AMINO-ACIDS

Since nearly all the known disintegration products of the main protein molecule are amino-acids, it may be of value to point out some of the distinguishing features of this class of bodies. They are soluble in water and easily diffusible. All the amino-acids, except glycine, derived from proteins are optically active, whereas those obtained by synthesis are inactive, or racemic, mixtures of the *d*- and *l*-forms.

The presence in the amino-acids of the basic radical NH_2 and of the acid group COOH lends to these bodies a double character. The monoamino-monocarboxylic acids, possessing neither acid nor alkaline reaction, are able in the presence of strong acids or bases to act either as base or acid. Thus, with hydrochloric acid, glycine forms glycine hydrochloride:

$$\begin{array}{l} CH_2NH_2HCl \\ | \\ COOH \end{array}$$

With bases these amino-acids form salt-like compounds such as potassium amino-acetate:

$$\begin{array}{l} CH_2NH_2 \\ | \\ COOK \end{array}$$

On account of the dual nature of the amino-acid molecule, these substances act as feeble electrolytes. Since they may act either as cations or anions, they are spoken of as *amphoteric* electrolytes (*v.* also p. 80)

Amino-acids also combine as acids and bases with one another. This power of combination much increases the difficulty of separating the constituents from a mixture of amino-acids. Amino-acids, which singly are sparingly soluble, are often soluble when in the presence of other amino-acids.

The diamino-acids, having two amino groups, are definitely basic in nature, while the dicarboxylic acids, with two carboxyl groups, are acids.

When an amino-acid is treated with formaldehyde a methylene compound is formed and the carboxyl group then has full acidic power and can be titrated.

$$\begin{array}{l} CH_2NH_2 \\ | \\ COOH \end{array} + H.CHO = \begin{array}{l} CH_2.N = CH_2 \\ | \\ COOH \end{array} + H_2O$$

Since each COOH group liberated corresponds to one NH_2 group fixed, this method is often used for estimating the amino-groups in any mixture.

An important property of amino-acids is that of combining with carbon dioxide to form carbamino-acids, *e.g.*:—

$$R.NH_2 + CO_2 = R.NH.COOH,$$

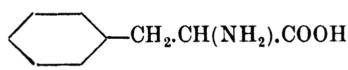
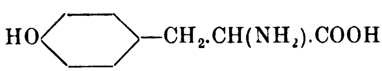
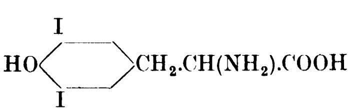
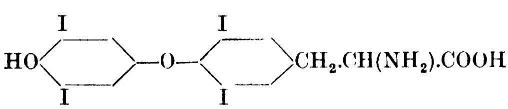
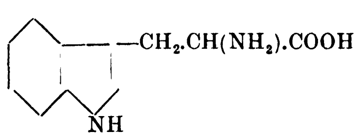
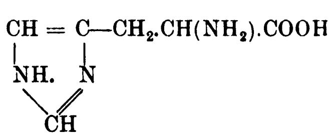
and this property is shared by certain proteins, which are thereby enabled to combine with carbon dioxide.

Since proteins are built up from a large number of amino-acids, it is not surprising to find that they likewise are amphoteric substances. The predominance of acid or basic properties will depend on whether the protein contains a surplus of dicarboxylic acids or of di-amino acids. It follows that the proteins can exist as salts with either acids or bases or in the free state. The factor which determines the condition of the protein is the hydrogen ion concentration. It has been found that the physical properties of protein solutions vary greatly with the hydrogen ion concentration and exhibit abrupt maxima or minima at a definite reaction which is characteristic of the protein. It seems probable that at this point—the *iso-electric point*—the protein is combined neither with acids nor with bases, but exists as a very feebly ionised free protein. On either side of the iso-electric point a highly ionised protein salt of

some sort will exist, the nature of which again depends on the hydrogen ion concentra tion. These characteristic changes of the protein molecule with the reaction of th medium promise to be very helpful in the interpretation of many difficult phenomena i connection with the physical chemistry of the cell.

The α-amino-acids which have been isolated from the products o hydrolysis of proteins are so varied that it may be a convenience to appenc a list of the physiologically important amino acids which have actually beer separated by hydrolysis of proteins. (The commonly used names are giver on the left.)

Monamino monocarboxylic (or 'Neutral') amino Acids.

Glycine.	$NH_2.CH_2COOH$	α-Amino acetic acid
d-Alanine.	$CH_3.CH(NH_2).COOH$	α-Amino propionic acid
l-Serine.	$CH_2(OH).CH(NH_2)COOH$	β-Hydroxy-alanine
d-Threonine.	$CH_3.CH(OH).CH(NH_2).COOH$	β-hydroxy-α-amino butyric acid
d-Norvaline.	$CH_3.CH_2.CH_2.CH(NH_2)COOH$	α-amino valeric acid
d-Valine.	$(CH_3)_2CH.CH(NH_2).COOH$	ββ-Dimethyl alanine
d-Norleucine.	$CH_3.CH_2.CH_2.CH_2.CH(NH_2).COOH$	α-amino caproic acid
l-Leucine.	$(CH_3)_2CH.CH_2.CH(NH_2).COOH$	β-Isopropyl alanine
d-Isoleucine.	$\begin{matrix}CH_3\\C_2H_5\end{matrix}\!>CH{-}CH(NH_2).COOH$	ββ-Methyl ethyl alanine
l-Cysteïne.	$HS.CH_2.CH(NH_2).COOH$	β-Thio-alanine
l-Cystine.	$\begin{matrix}S.CH_2.CH(NH_2).COOH\\ \vert \\ S.CH_2.CH(NH_2).COOH\end{matrix}$	Dicysteine
l-Methionine.	$CH_3.S.(CH_2)_2CH(NH_2)COOH$	γ-Methylthiol — α-Amino butyric acid
l-Phenyl alanine.		Phenyl alanine
l-Tyrosine.		*p*-Hydroxyphenyl alanine
3, 5, Diiodotyrosine.		
Thyroxine.		
l-Tryptophan.		β-Indol alanine
l-Histidine.		β-Iminazol alanine

Diamino- (or Basic Amino-) Acids

Ornithine. $NH_2(CH_2)_3CH(NH_2).COOH$ αδ-Diamino-valeric acid

Citrulline. $NH_2.CO.NH.(CH_2)_3.CH(NH_2).COOH$

d-Arginine. $NH—(CH_2)_3—CH(NH_2)COOH$ α-amino-δ-guanidino-valeric acid
$|$
$C = NH$
$|$
NH_2

d-Lysine. $NH_2(CH_2)_4CH(NH_2).COOH$ α-Diaminocaproic acid

Dicarboxylic (or Acidic Amino-) Acids

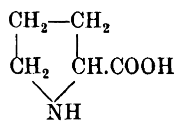
l-Aspartic Acid. Aminosuccinic acid
$CH_2.COOH$
$|$
$CH(NH_2).COOH$

d-Glutamic Acid. α-Aminoglutaric acid
$CH_2.COOH$
$|$
CH_2
$|$
$CH(NH_2).COOH$

d-Hydroxyglutamic Acid. α-Amino, β-hydroxyglutaric acid
$CH_2.COOH$
$|$
$CHOH$
$|$
$CH(NH_2).COOH$

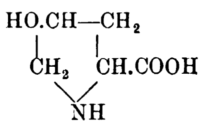
"Condensed" Amino-Acids

l-Proline.



α-pyrrolidine carboxylic acid.

l-Hydroxyproline.



γ-hydroxy-α-pyrrolidine carboxylic acid

Several of the chemical tests for proteins depend on the presence of specific amino-acids; for example, the Millon's reaction on the presence of tyrosine, the glyoxylic test on the presence of tryptophan, &c.

Under the action of bacteria amino-acids may undergo a process of deamination which may be accompanied by simple hydrolysis or by reduction. In the former case an amino-acid may be converted into an hydroxyacid, in the latter case into a fatty acid.

On the other hand, some bacteria may split off carbon dioxide from the amino-acids and yield amines. Thus tyrosine becomes *p*-hydroxyphenylethylamine, a substance having marked physiological effects. Phenylalanine $C_6H_5.CH_2.CH.NH_2.COOH$, becomes phenylethylamine $C_6H_5.CH_2.CH_2.NH_2$.

Other Constituents of the Protein Molecule

When we add together the total amino-acids obtainable by the acid disintegration of any given protein, a considerable proportion of the original protein is often unaccounted for. Much of this remainder may represent analytical loss, but some of it may consist of substances not as yet identified.

One substance sometimes present is glucosamine. Proteins belonging to the class of mucoids yield on acid hydrolysis from 16 to 30 per cent. glucosamine. Egg albumin, serum albumin and serum globulin all contain in their molecules a complex carbohydrate consisting of glucosamine and mannose.*

The Building up of the Protein Molecule

The amino-acids are present in different proportions in the individual proteins, so that in many cases a large number of identical amino-acid groups must be present in the protein molecule, with smaller numbers of other groups. One method of linkage of amino-acids to form larger molecules is by condensation of a COOH of one amino-acid and an NH_2 group of another to form the linkage :

$$-\ CO.NH\ -$$

which is now called the *peptide linkage.* It is generally believed that, in this manner, molecule can be added to molecule until finally the gigantic molecule of a protein results. In such molecules there is believed to be a regular pattern of amino-acids, each of which recurs with constant frequency at a particular place in the chain.†

This theory of the constitution of proteins is supported by the fact that only a small proportion of the NH_2 groups present in the separated amino-acids exist free in the protein molecule. The linking of the amino groups must therefore take place by NH groups. Synthetic experiments have fully confirmed this hypothesis. Fischer, and latterly Bergmann,‡ have devised a number of ingenious methods for combining together two or several amino-acids of any character. Thus from two molecules of glycine we may obtain the compound glycyl glycine, as follows :

$$NH_2.CH_2.COOH + HNH.CH_2.COOH =$$
$$NH_2.CH_2.CO.NH.CH_2.COOH + H_2O$$

These compounds were designated *peptides* by Fischer. He distinguished di-, tri-, tetra, &c., or *polypeptides* according to the number of individual amino-acids taking part in the formation of the compound, *e.g.* alanyl leucine, glycyl tyrosine, dialanyl cystine, dileucyl cystine, leucyl pentaglycyl glycine, and so on. The last named would be built up out of one molecule of leucine, and six molecules of glycine. Abderhalden built up a molecule containing eighteen constituent amino-acids. These polypeptides are still very far below the proteins in molecular size, and proteins have not been made artificially. The higher polypeptides closely resemble the peptones. Most of them, even if derived from relatively insoluble amino-acids, are soluble in water, insoluble in alcohol. They dissolve in mineral acids and in alkalies, with the formation of salts, and have a bitter taste. The higher members of the series give certain reactions, such as the biuret reaction, which are regarded as characteristic of peptones, and like the latter are precipitated by phosphotungstic acid. Some are split up by certain proteinases,§ and by erepsin, into amino-acids.

Thus in the building up of the protein molecule there is an almost unlimited capacity for coupling up of numerous amino-acid groups, the link in each case being the nitrogen. Of the two or more possible optical isomers of

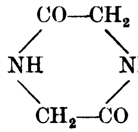
* Rimington. *Biochem. J.*, 1929, **23**, 430.
† Bergmann and Niemann. *J. Biol. Chem.*, 1937, **118**, 301 ; 1938, **122**, 577.
‡ Bergmann. *Science*, 1934, **79**, 439. Bergmann and Zervas. *J. Biol. Chem.*, 1936, **113**, 341.
§ Bergmann, Fruton and Fraenkel-Conrat. *J. Biol. Chem.*, 1937, **119**, 35.

each amino-acid containing more than two carbon atoms, only one is made use of for this purpose.

A still further flexibility in its reactions is conferred on the protein molecule by intramolecular rearrangement occurring with great readiness in its component molecules. If we take the simplest member of the class of dipeptides, glycyl glycine, its usual formula is :—

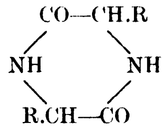
$$NH_2CH_2CO - NH.CH_2.COOH$$

But union between two amino-acids readily occurs by condensation to form a *diketopiperazine.* Thus from glycine we get, by elimination of water, the ring compound,



Diketopiperazine or glycine anhydride.

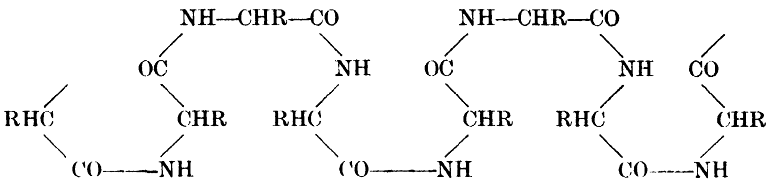
and, similarly, from any other two amino-acid molecules we get corresponding diketopiperazines of the type .



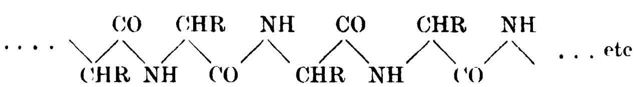
There are grounds for the belief that such linkages may also occur in the union of the amino-acids to form the protein molecule. The fact that pepsin will act on proteins, but not on polypeptides, is a further indication that the peptide structure may not be the only type of linkage by which the constituent amino-acids are assembled in the protein molecule. If we consider that perhaps some hundred of the amino-acid groups may go to making up a single protein molecule, it is possible to form some conception of the enormous variability in structure possible to such a compound.

The study of the ultrastructure of proteins by the use of X-ray spectra and by other physical methods, such as the study of thin films, orientation in electric fields, etc., gives another viewpoint from which to study the architecture of their molecules. These investigations show two types of molecular structure, *fibrous* and *globular.*

Among the fibrous proteins are those of myosin, keratin, and fibroin, present respectively in muscle, hair and silk. In these we have essentially a long polypeptide chain. In the normal state the chain is kinked owing to electrostatic attraction between side-chains, *e.g.* in α-keratin :—



α-keratin



β-keratin (stretched)

but when stretched the chain becomes more regular (β-keratin), and if heated in the stretched state this form becomes permanent ("permanent waving"). The myosin of muscle is probably similar.

In the globular protein molecules, the kinking is exaggerated, so that the molecule folds on itself in a regular pattern. Such proteins are insulin, pepsin, lactoglobulin and hæmoglobin. According to Wrinch,* regular geometrically constructed molecules may

* WRINCH. *Proc. R. S.*, 1937 (A), **160**, 63 ; **161**, 505.

result (cyclol structure), containing 6, 18, 30, 42, 54, 66, 72, 90 or more amino-acids, which ultimately form a globular structure. In these molecules it is assumed that some of the peptide linkages are tautomerised to – C(OH) : N – linkages.

THE CONSTITUTION OF DIFFERENT PROTEINS

Nearly all the proximate constituents of the main protein molecule, so far as we know, are amino-acids. Of these the following have been isolated,

	Serum albumin.	Egg albumin	Edestin (hemp seeds).	Gliadin.	Caseinogen	Globin.	Salmin.	Sturin.	Gelatin.	Keratin (from horse hair).	Zein.
Glycine.	0	0	3 8	0 9	0·45	0	0	—	25·5	4·7	0 0
Alanine	2·7	8·1	3·6	2 7	1·85	4·2	—	—	8·7	1 5	9·8
Serine .	0 6	—	0 33	0·12	0·5	0·6	7 8	—	0 4	0·6	1·0
Valine .	—	—	present	0·3	7 93	—	4 3	—	—	0 9	1·9
Leucine	20·0	7 1	20 9	6 0	9 7	29·0	0	—	7·1	7·1	25·0
Proline.	1·0	2 25	1·7	2·4	7·63	2·3	11·0	—	9 5	3·4	9·0
Hydroxyproline .	—	—	2 0	—	0·23	1 0	—	—	14 1	—	—
Glutamic acid	7 7	8 0	6 3	36·5	21·77	1·7	—	—	5 8	3·7	31·3
Aspartic acid.	3 1	1 5	4·5	1·3	1·77	4·4	—	—	3·4	0·3	1·8
Phenyl alanine	3 1	4·4	2·4	2 6	3·88	4·2	—	—	1·4	0	7·6
Tyrosine	2·1	1·1	2 1	2 4	4·5	1·5	—	—	0 01	3·2	5·7
Tryptophan .	present	present	present	1·0	1·50	present	—	—	—	—	0·0
Cystine.	2 3	0·2	0 25	0·25	?	0·3	—	—	—	10+	0 8
Lysine .	—	2·15	1 0	0	7·62	4 3	0	12 0	5 9	1·1	0·0
Arginine	—	2·14	11·7	3 4	3·81	5·4	87·4	58·2	8·2	4·5	1·8
Histidine	—	—	1·1	1·7	2·5	11 0	0	12 9	0·9	0 6	0·8
β-hydroxy-glutamic acid .	—	—	—	—	10 0	—	—	—	0 0	—	2·5

namely, glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, phenyl alanine, glutamic acid, β-hydroxyglutamic acid, aspartic acid, tyrosine, tryptophan, cystine, methionine, lysine, histidine, and arginine.

The question now arises whether all the varieties of protein owe their peculiarities to the presence of different amino-acids, or whether the greater number of the amino-acids above mentioned are present in all proteins, the differences between the latter being determined by differences in the arrangement and relative amounts of these proximate constituents. A large number of analyses of various proteins have been made by Abderhalden, Osborne, Dakin and others, utilising the methods for the isolation of amino-acids devised by Fischer and by Dakin. The constitution of some representative proteins as determined in this way is given in the Table above.

These results show that all the proteins contain a very considerable proportion of the total number of amino-acids which have as yet been isolated from acid digests of proteins. The differences in various proteins cannot therefore be determined by qualitative differences in their constituent molecules, but must depend on the relative amounts of the amino-acids which are present and on their arrangement in the whole molecule. As regards relative amounts of amino-acids we find very striking variations. Thus glutamic acid, which forms 8 per cent. of egg albumin and only 1·7 per cent. of globin (derived from hæmoglobin), amounts to 36·5 per cent. in gliadin, the protein extracted from wheat flour. Striking differences are also noticeable in the relative proportions of the diamino-acids, the so-called hexone

bases. Whereas in casein they form about 12 per cent. of the total molecule, in globin they form about 20 per cent.; and in the protamines, salmin and sturin, about 85 per cent. of the total molecule consists of these bodies. On this account the two last-named proteins have a strongly basic character. From these figures it is evident also that certain of the amino-acids must occur many times over in the protein molecule. Thus in globin, if we assume the presence of one tyrosine molecule, there must be at least thirty-two leucine and ten histidine molecules. On these data the minimal molecular weight of hæmoglobin would come at about 17,000.

The Molecular Weight of Proteins. We may arrive at an approximate idea of the minimum size of the protein molecule in various ways, though all our calculations are apt to be vitiated by the difficulty of obtaining a preparation which is chemically pure, and by the ease with which molecules of the size which we must assume for proteins aggregate together or form adsorption combinations in varying proportions with other substances. Some idea of the molecular complexity represented by these weights may be gained by writing out the possible empirical formulæ of the various proteins, *e.g.*:

Egg albumin	$C_{204}H_{322}N_{52}O_{66}S_2$
Hæmoglobin	$C_{712}H_{1130}O_{245}S_2Fe$

On the simplest empirical formula, therefore, most of the proteins have molecular weights of several thousand, *e.g* hæmoglobin is at least 17,000. The application of physical methods, such as the rate of sedimentation at high-speed centrifugalisation, diffusion, viscosity, etc., shows that under most conditions these molecules are aggregated into larger complexes, which, however, can be broken up by altering the conditions. Most of these composite molecules have a molecular weight, according to Svedberg, which is a multiple of 17,600, as *e.g.*:—

Protein	Molecular weight by sedimentation Velocity	M (calc.)
Lactalbumin . .	17,500	17,600
Lactoglobulin . .	41,800	2 × 17,600
Insulin . . .	40,900	2 × 17,600
CO-Hæmoglobin .	69,000	4 × 17,600
Serum globulin . .	167,000	8 × 17,600
Hæmocyanin (*Helix*) .	6,630,000	384 × 17,600

(*v.* also p. 77).

THE CLASSIFICATION OF PROTEINS

At the present time it is impossible to make any classification of proteins based on their constitution, and we have therefore to use an arbitrary classification, based chiefly on the solubilities of the various proteins. We shall here only indicate the characters of the main groups.

(1) THE PROTAMINES. These occur only in combination. They are obtained from the ripe spermatozoa of certain fishes, where they are in combination with nucleic acid. They are of relatively low molecular weight, *e.g.* 2,000, and are characterised by the very large amount of diamino-acids in their molecule. A small proportion of mono-amino-acids may also be obtained from their disintegration (*v.* Salmin, Table, p. 50). They possess strongly basic characters and form well-marked salts, *e.g.* sulphates, as well as double salts with platinum chloride. They contain no sulphur and do not coagulate on heating.

(2) HISTONES. This class of proteins, like the protamines, only occurs in

combination with other substances, such for instance as nuclein and hæmatin. They may be obtained from red blood corpuscles, where they form the globin part of the hæmoglobin molecule, or from the thymus gland. The histones are precipitated from their watery solutions by addition of ammonia, but are soluble in excess of this reagent. In the presence of salts they are coagulated on boiling. The most characteristic feature is the high proportion of diamino-acids contained in their molecule.

(3) ALBUMINS. These are soluble in water and are precipitated by complete saturation with ammonium sulphate or zinc sulphate.

Egg Albumin forms the greater part of the white of egg. It gives the ordinary protein tests, coagulates on heating at about 75° C., and is precipitated from its solutions if shaken with a drop of dilute acetic acid in excess of ether. It is lævo-rotatory, its specific rotatory power being — 35·5°.

Serum Albumin occurs in large quantities in the blood plasma, serum, lymph, and tissue fluids of the body. It coagulates at 75° C., and is distinguished from egg albumin by its greater specific rotatory power, — 56°, and by the fact that it is not precipitated by ether and acetic acid. Some vegetable proteins belong to this class, *e.g.* the *leucosin* of wheat.

(4) GLOBULINS. These bodies are insoluble in water and require the presence of a certain amount of neutral salt to dissolve them. They are precipitated from their solutions by complete saturation with magnesium sulphate or by half-saturation with ammonium sulphate. Important members of this class are :

Crystallin, obtained from the crystalline lens by passing a stream of carbon dioxide through an aqueous extract of this body.

Serum Globulin, a constituent of blood plasma and blood serum.

Fibrinogen, which occurs in blood plasma and is converted into fibrin when the blood clots.

Myogen, a normal constituent of muscle.

Midway between these two groups may be placed the muscle protein, *myosin* (or *myosinogen*) which, though soluble in water, resembles the globulins in the ease with which it is precipitated by the addition of neutral salts.

Proteins allied to this class form an important constituent of plants, and are found in large quantities in many seeds used as articles of food. Prominent members of the vegetable globulins are the *edestins*, from hemp, cotton, and sunflower seeds, *zein* from maize, *legumin* from beans.

(5) GLIADINS, contained in cereals, and soluble in dilute alcohol.

(6) GLUTELINS, also obtained from cereals, and soluble in weak alkalies.

(7) DERIVATIVES OF PROTEINS. When proteins are heated with acids, or acted on at the body temperature by certain enzymes, *e.g.* pepsin, trypsin, or papain, they undergo hydrolysis. This action, when carried to its end, results in the production of the amino-acids which we have already dealt with.

These hydrolytic changes proceed by a series of stages, so that the intermediate products still present many of the protein reactions. These derived proteins are divided into three groups, *metaproteins*, *proteoses* and *peptones*. The formation of these intermediate products is especially marked with the proteolytic enzymes. Pepsin, together with hydrochloric acid, for example, only breaks down the protein molecule into smaller, but still quite large molecules, the proteoses and peptones. It does not liberate any amino-acids. Trypsin gives rise to proteoses, peptones and also amino-acids. In both cases, the peptide linkages are attacked, since equal numbers of NH_2 and COOH groups become free. The action of these enzymes on proteins, in fact, results in the production of a number of derivatives of

progressively diminishing molecular weight and complexity by a hydrolytic process :—

$$\ldots R.CO.NH.R \ldots + H_2O \longrightarrow \ldots R.COOH + NH_2.R \ldots$$

The protein molecule is distinguished by the variety of the groups which enter into its formation, and this renders possible a great variety of intermediate products. Thus a protein molecule may consist of the amino-acids, A, B, C, D, F, G, H, &c. When hydrolysis occurs it may result in the immediate splitting off, say, of group A, while the residue breaks up into a series of proteoses whose composition may be represented as ABF, ABC, DFG, BDEF, &c. With further hydrolysis these groups are broken into still smaller ones, and the penultimate stages of the hydrolysis will be polypeptides.

No sharp dividing line can be drawn between the metaproteins, proteoses, peptones, and polypeptides. We have at present no satifactory means of isolating the different members of these groups. Their classification is, therefore, a conventional one, depending on their solubilities and their precipitability by neutral salts. Both proteoses and peptones give reactions common to all proteins and, like these, are precipitated by such reagents as mercuric chloride, or phosphotungstic acid. They give the biuret reaction. Their solutions can be boiled without coagulation. Many of them may be thrown down from their solutions by alcohol.

(*a*) *Metaproteins.* These are amongst the early products of hydrolysis of the proteins and are little less complex than the proteins from which they are derived.

Acid Metaprotein is formed by the action of dilute acids on any proteins. If a weak alkali be added so as nearly to neutralise the solution of acid metaprotein, this latter is precipitated.

Alkaline Metaprotein is formed by the action of strong alkalies on any protein, or by adding alkali in excess to a solution of acid metaprotein. It is precipitated on neutralisation of its solution.

(*b*) *Proteoses, e.g. albumose* from albumin, *caseose* from casein, *elastose* from elastin. All of these are precipitated from their solutions on saturation with ammonium sulphate. In the presence of a neutral salt they give a precipitate on the addition of nitric acid. All, with the exception of hetero-albumose, are soluble in water, and all are soluble in weak salt solutions or dilute acids or alkalies. They are slightly diffusible through animal membranes.

(*c*) *Peptones, e.g.* fibrin peptone, gluten peptone. These are all soluble in water, diffuse fairly readily through animal membranes, and give reactions similar to those of the albumoses. From the latter they are distinguished by the fact that they are not precipitated on saturation of their solutions, either in acid or alkaline reaction, with ammonium sulphate. Many of them are soluble in alcohol.

(*d*) *The polypeptides* resemble peptones, but are more readily diffusable.

(8) Conjugated Proteins. Various complex bodies which play an important part in building up cells, and in the vital processes of the body, make up this group of compounds. They resemble one another only in the fact that in each of them a protein radical is combined with some other body, often spoken of as the *prosthetic* group.

(*a*) *The Phosphoproteins.* In this class may be included a number of substances of very diverse properties, which, however, resemble one another in containing phosphorus as an integral part of their molecule. The phosphoproteins have markedly acid characters. They are insoluble in water, easily

soluble in alkalies, from which the original body is thrown down again on addition of acid. Their solutions in alkali are not coagulated by heating. To this class belong *caseinogen*, the chief protein of milk, and *vitellin*, the main protein in the yolk of egg. The vitellins are generally associated with a large amount of lecithin. The phosphoproteins differ from the nucleoproteins, which also contain phosphorus, in that they are readily decomposed by dilute caustic alkali with the liberation of phosphoric acid, and do not contain purine bases.

(*b*) *Chromoproteins.* Of this class, consisting of a colouring matter combined with a protein, the most important is *hæmoglobin.* This substance, which is the red colouring matter of the red corpuscles of the blood, is composed of the protein, globin, united with from 4–5 per cent. of an iron-containing body, hæmatin $C_{32}H_{32}N_4O_4Fe$.

(*c*) *The Nucleoproteins.* These are formed by the combination of a phosphorised organic acid, *nucleic acid*, with a protein which is usually one of the basic histones or protamines. If a nucleoprotein be subjected to gastric digestion, a large amount of the protein goes into solution as proteose or peptone, leaving an insoluble remainder. This precipitate still contains a protein group, and is spoken of as *nuclein.* From the latter nucleic acid can be split off by heating with strong acids or by trypsin. The relation of the two portions of the nucleoprotein may be represented :—

Nucleoprotein

Protein — Nuclein

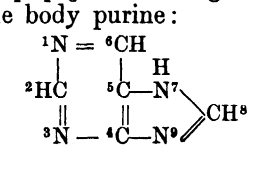
Protein (generally histone or protamine) — Nucleic acid

The nucleoproteins are soluble in water, salt solutions and dilute alkalies. They have acid characters and are precipitated by acids. The nucleins, on the other hand, are insoluble in water and salt solutions, but are dissolved by dilute alkalies. The nucleins and nucleoproteins form the chief constituents of cell nuclei. They may therefore be prepared from the most diverse organs. The heads of spermatozoa consist entirely of nuclein. Nucleoproteins may be obtained from organs rich in cells, such as the thymus and the pancreas, and from nucleated red blood corpuscles.

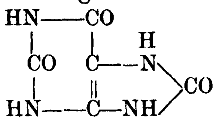
Nucleic Acid. By hydrolysis, the nucleic acid may be broken up into its proximate constituents. These differ slightly according to the source of the nucleic acid. Whatever the source, the disintegration products always contain phosphoric acid, purine bases, pyrimidine bases, and a pentose.

(*1*) Phosphoric Acid. The proportion of phosphorus in the nucleic acids is about 10 per cent. It represents, so to speak, the combining link for the groups contained in the nucleic acid molecule.

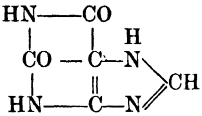
(*2*) The Purine Bases. Among the products of disintegration of nucleic acid we find constantly one of the bases *adenine*, $C_5H_5N_5$, and *guanine*, ($C_5H_5N_5O$). These substances, with the products of their oxidation, *xanthine*, $C_5H_4N_4O_2$, *hypoxanthine*, $C_5H_4N_4O$, have long been known to be closely allied to uric acid, $C_5H_4N_4O_3$. According to Fischer they can be all regarded as derivatives of the body purine:



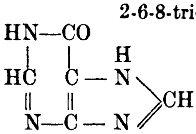
The relation of xanthine, hypoxanthine, guanine, and adenine to uric acid is shown by the following formulæ :



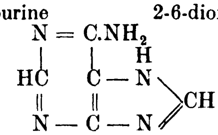
Uric acid
2-6-8-trioxypurine



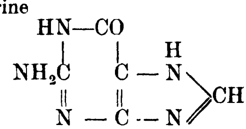
Xanthine
2-6-dioxypurine



Hypoxanthine
6-oxypurine



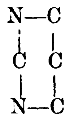
Adenine
6-amino-purine



Guanine
2-amino 6-oxypurine

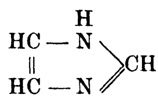
Closely allied to this group of bodies are the active constituents of tea, coffee, and cocoa, namely *caffeine*, which is *trimethyl dioxypurine*, and *theobromine*, which is *dimethyl dioxypurine.*

From the structural formulæ given it will be seen that the purine ring contains two nuclei. The nucleus :



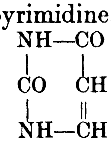
is spoken of as the *pyrimidine nucleus.*

The other is the *iminazol* ring which we have met with already in histidine:

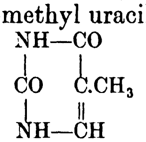


Besides the purine bases proper, we find among the disintegration products of nucleic acid a series of bases derived from the pyrimidine ring. These are uracil, thymine, and cytosine.

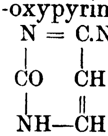
Uracil, 2-6-dioxy-pyrimidine,



Thymine, 5-methyl uracil,



Cytosine, 6-amino-2-oxypyrimidine,



(*3*) In addition to these two groups of compounds, the nucleic acids also contain a pentose group. In the animal nucleic acids this is a desoxypentose ($C_5H_{10}O_4$), viz. *d*-2-ribodesose,* but in plant nucleic acids and in the mononucleotides of animal tissues, *e.g.*, adenylic acid, the carbohydrate group is the ribofuranose, γ-ribose,† of empirical formula $C_5H_{10}O_5$. In general, vegetable and animal nucleic acids comprise respectively the following units :

Animal.	*Vegetable.*
Phosphoric acid.	Phosphoric acid.
Ribodesose.	Ribose.
Adenine and guanine.	Adenine and guanine.
Cytosine and thymine.	Cytosine and uracil.

The researches of Levene and of others have thrown light on the manner of combination of these groups in the molecule of nucleic acid. It appears that

* Levene, Mikeska and Mori. *J. Biol. Chem.*, 1930, **85**, 785.
† Levene and Tipson. *J. Biol. Chem.*, 1932, **94**, 809.

each purine or pyrimidine group can combine with a pentose to form what is called a '*nucleoside*,' *e.g.* :—

$$\underset{\text{(Adenine)}}{C_5H_4N_5}\text{—}\underset{\text{(Ribose)}}{C_5H_9O_4}$$ is the nucleoside *adenosine*.

Each nucleoside can be then attached to a molecule of phosphoric acid, this complex being named a '*nucleotide*' and representing a nucleic acid in its simplest form, *e.g.* :—

$$\underset{\text{(Adenine)}}{C_5H_4N_5}\text{—}\underset{\text{(Ribose)}}{C_5H_8O_3}\text{—}\underset{\text{(Phosphoric acid)}}{PO_3H_2}$$ is the (mono) nucleotide *adenylic acid.*

In general, nucleic acids are *tetranucleotides*, in which four mononucleotides are linked together, possibly through the phosphoric acid molecule of one and the carbohydrate of the next. Thus thymus nucleic acid, a tetranucleotide, would have the structure

```
                                  Phosphoric acid—Pentose—Guanine
                                                    |
                    Phosphoric Acid—Pentose—Cytosine
                                      |
      Phosphoric Acid—Pentose—Thymine
                        |
Phosphoric Acid—Pentose—Adenine
```

The degradation of nucleoprotein may be rendered clearer by the following scheme:

```
            Animal
         Nucleoprotein
               ↓
         Nuclein + Protein
               ↓
         Nucleic acid + Protein
               ↓
      4 Mononucleotides
               ↓
      4 Nucleosides + 4 Phosphoric acid
               |
   ----------------------------------------
   |                 |                    |
4 Desoxy-      Guanine and         Thymine and
 Ribose          Adenine             Cytosine
```

(*d*) *The Glycoproteins.* In the glycoproteins the prosthetic group is represented by a carbohydrate radical, generally containing nitrogen, such as glucosamine or galactosamine. Serum albumin and globulin both contain a carbohydrate consisting of glucosamine and mannose.* The glycoproteins proper are split into their two constituents, protein and carbohydrate, on boiling with dilute mineral acids. The carbohydrate prosthetic group is a tetrasaccharide, derived *e.g.* from two acetylhexosamine molecules joined together by two glucuronic acid molecules. The hexosamine called glucosamine gives a tetrasaccharide called *mucoitin*, while galactosamine yields the tetrasaccharide called *chondroitin*—they occur respectively as mucoitin and chondroitin sulphuric acids. Heparin is a derivative of some such compound as chondroitin sulphuric acid.

The glycoproteins are divided into the two main groups, *viz.* : mucins and mucoids.

The *mucins* are present in the slimy secretion of the mucous membranes and which is secreted either by goblet cells or by mucous glands. They may be precipitated from their solutions by the addition of acids, and redissolve

* RIMINGTON. *Biochem. J.*, 1931, 25, 1062.

on addition of alkalies. They are not coagulable by heat. They give the various typical protein tests, such as the xanthoproteic, Millon's, the biuret reaction, and so on. Prolonged boiling with acids splits the molecule, with the production of acid metaprotein and mucoitin sulphuric acid.

The *mucoids* include a number of substances which may be extracted from various tissues by the action of weak alkalies, *e.g.* from tendons, bone and cartilage. Another example of this class of mucoids is *ovomucoid,* which is a constituent of egg-white. A similar body has been prepared from blood serum. The best studied example of this group is the *chondromucoid* which, with collagen, forms the ground substance of cartilage. Chondromucoid on hydrolysis yields a protein, and both mucoitin and chondroitin sulphuric acids.

(9) THE ALBUMINOIDS OR SCLERO-PROTEINS. Under this heading are grouped a number of diverse substances which play an important part in building up the framework of the body. They play the part in the animal economy which in the vegetable kingdom is filled by the celluloses, lignin, the pentosans, &c. Their value as skeletal tissue seems to be determined by their insoluble character, but on this account it is practically impossible to purify them; we can simply take the residue of a tissue which is left after extraction of the soluble constituents. When broken down by the action of strong acids, they yield disintegration products which resemble those derived from other proteins.

Collagen forms the main constituent of white fibrous tissue and the ground substance of bone and cartilage. Under the action of acids or when subjected to prolonged boiling with water it is converted into *gelatin,* which forms a colloidal solution setting to a jelly when cold. When subjected to acid hydrolysis it gives a series of amino-acids from which tyrosine and tryptophane are wanting. On the other hand, there is a preponderance of glycine and phenyl alanine. Gelatin is hydrolysed by gastric juice or by pancreatic juice, whereas collagen is unaffected by the latter.

The *Keratins* are produced by the modification of epithelial cells, and form the horny layer of the skin as well as the main substance of hairs, wool, nails, hoofs, horns and feathers. They are distinguished by their insolubility in water, dilute acids or alkalies, and if eaten pass through the alimentary canal unchanged. Although differing in their elementary composition, they are all distinguished by the very large amount of sulphur present in their molecule. The greater part of this sulphur is in the form of cystine, of which keratin contains as much as 10 per cent.

Neurokeratin, which forms the basis of the neuroglia of the central nervous system, is grouped with the keratins. It is extracted from nervous tissues by boiling these with alcohol and ether, and then submitting the tissue to tryptic digestion which leaves the neurokeratin unaffected.

Elastin forms the elastic fibres of the connective tissues. Elastin is insoluble in water, alcohol or ether, or in dilute acids and alkalies. It is slowly dissolved on *prolonged* treatment with gastric juice, but is practically unaffected in the alimentary canal.

Other members of this group are *fibroin,* which forms the main substance of silk; *spongin,* the horny framework of sponges; *conchiolin,* the ground substance of shells: and perhaps the amyloid substance which we have already mentioned in connection with the mucoids. All these sclero-proteins present considerable differences in their qualitative and quantitative content of amino-acids.

SPECIFICITY OF PROTEINS. Although certain proteins may appear to be identical so far as ordinary chemical and physical properties, and even so far as chemical analysis is concerned, they are not necessarily identical in their biological properties. For

example, casein from cow's milk and casein from goat's milk, or egg albumin from hen or duck, have certain specific properties by which they are distinguished from one another. This can be shown by *anaphylactic reactions* (*v.* Chapter XXXVII.). Thus, if a guinea-pig is sensitised by injections of hen-egg albumin, administration of egg albumin from another species is not followed by any effects, whereas an extremely small injection of the hen albumin promptly causes death from anaphylactic shock. There is thus a species specificity with proteins which is not seen with other biochemical constituents.

Dudley and Woodman * showed that this is in all probability due to the fact that in the different species there are slight differences in the *arrangement* of the constituent amino-acids in the protein molecule. Thus if in one species we have the amino-acids A, B, C, D, E . . . arranged in that order, we should have a stereochemically different molecule if the arrangement were B, D, A, C, E . . . . These species differences are of great importance in connection with the subject of immunity; they also explain why, in the construction of protein in the body, the protein taken as food must first be thoroughly broken down into its constituent amino-acids.

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* DUDLEY and WOODMAN. *Bioch. Journ.*, 1915, **9**, 97.

C. BIOPHYSICAL PRINCIPLES *

CHAPTER V

THE ENERGY OF MOLECULES IN SOLUTION

THE ultimate source of the energies displayed by the animal organism is the chemical energy of the substances taken in as food. In the living cell the chemical energy of the food may undergo conversion into other forms, *e.g.* heat, work, electrical difference of potential, or it may be used for the production of other chemical substances possessing perhaps as much potential energy as, or more than, the foodstuffs themselves.

Water forms from 70 to 95 per cent. of the mass of protoplasm. No substance introduced into the protoplasm has any chemical influence on it unless it be soluble, and the first stage in the preparation of foodstuffs for assimilation always consists in a process of solution. It follows that all the energy with which we have to deal is the energy of *molecules in watery solution.*

DIFFUSION. When a substance (solute) is dissolved in a liquid (solvent), we may regard the molecules of both solute and solvent as being in continual movement within the liquid, very much as the molecules of a gas are in continual motion. In consequence of this state of movement solutes travel by diffusion from places where they are more concentrated to parts where they are more dilute, until finally a uniform concentration obtains throughout the solution. Thus, if a vessel be half filled with a 10 per cent. solution of cane sugar, and be then filled up carefully with water, so as to form a layer on the heavier sugar solution, the sugar gradually diffuses up into the water, and after some weeks there will be the same concentration of sugar throughout all parts of the fluid. Carried out in this way, the process of equalisation is a slow one, but when aided by the production of currents, or when only thin films of liquids are concerned, spread by diffusion may be effected in quite short periods of time.

Dialysis.—Many animal or vegetable membranes, and some artificial membranes, such as cellophane or collodion, allow water and solutes to pass freely through them, and this permeation is called *dialysis.* This fact is utilised as a means of separating substances in true solution from other substances not so dissolved, since the latter will not dialyse out into water placed on the other side of such a membrane. In the process of dialysis, however, not only does solute pass out into the water, but water also passes inwards into the solution, so that this tends to become diluted. These phenomena are easily explained if we remember that molecules of solvent and solute are in constant movement, and if we regard the solution as being weaker in solvent than the pure solvent is. Thus, to consider a strong cane sugar solution and water or a weak cane sugar solution, the former consists of a high concentration of sugar molecules and a lowered concentration of water molecules, while the latter is a high concentration of water molecules and a low (or non-existent) concentration of sugar molecules. Consider that we have a vessel with two such 'solutions,' A strong and B weak (or

* As with the chapters on Structural and Biochemical Principles, the present chapter can hope to be no more than an outline indicative of the main aspects of the important branch of biology called Biophysics, for further details of which special treatises must be consulted.

B = water), at the same level, and separated by a membrane *m*, permeable to both sorts of molecule, as in Fig. 14 (I). Then solvent and solute can both move in either direction, but because solute is more concentrated in A and solvent more concentrated in B, there will be a greater passage of solute into B, and of solvent into A, than in the reverse direction. Ultimately, when the two solutions have become uniform in composition, A by

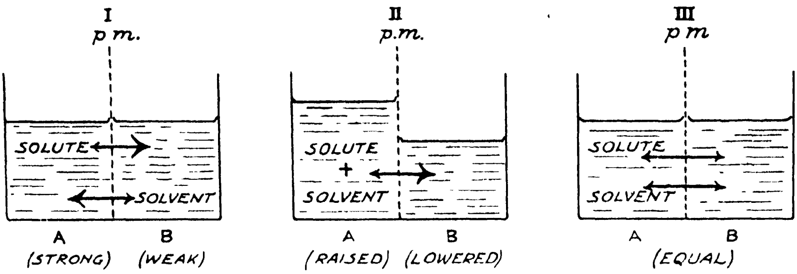

FIG. 14.

losing solute and gaining water, B by gaining solute and losing water, there would at first be a raised level of solution in A as compared with B, Fig. 14 (II). But this difference of hydrostatic pressure, since the membrane is freely permeable to both solvent and solute, causes fluid of the uniform composition to flow from A to B, until it reaches the same level, and true equilibrium is now reached (Fig. 14 (III)).

Semi-permeable Membranes. Membranes may be of different degrees of permeability, according to the sizes of their structural pores, so that some solutes may be let through, but not others of larger molecular size. A special case arises when only the solvent can pass through, while all solutes are held back. This is called a *semi-permeable membrane*. If we consider the case of a solution separated from its solvent by such a membrane, it is clear that if we start with pure solvent on one side, equilibrium can never be established in the sense that the solutions become of the same composition on both sides of the membrane, because ultimately all the solvent could be absorbed into the solution, since no solute would pass out.

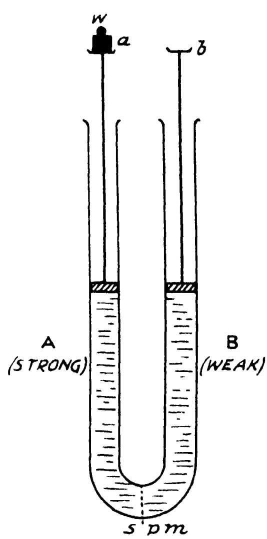

FIG. 15.

OSMOTIC PRESSURE

This attraction of solvent to a stronger from a weaker solution is known as *osmotic pressure*, or, more correctly, as *osmotic water attraction*. Consider the case of a strong and a weak solution (or pure solvent) separated by a semi-permeable membrane, *s.p.m* in the vessel A, B (Fig. 15), each limb of which is provided with a watertight piston, bearing a scale-pan *a*, *b*. Left to itself, solvent will pass from B to A, owing to the osmotic pressure or osmotic water attraction of the solution in A. If a weight W, be placed on the pan *b*, the rate at which water passes into A will be accelerated, because solvent will be driven through the *s.p.m* by the pressure put upon it. If, instead, the weight be placed on the pan *a*, as in the figure, the rate at which solvent enters A will be reduced, and if the weight placed on *a* be

heavy enough, solvent can be driven out of A into B, against the osmotic water attraction of the solution in A. If the weight on *a* be just sufficient to prevent water either entering A or leaving it, a state of equilibrium is reached, the weight just counteracting the osmotic pressure of the solution, and so giving a measure of its magnitude.

Direct experiment has shown that the osmotic pressure exerted by a solute in a given volume of solvent is the same as the pressure the same weight of solute would exert when in a state of gas occupying the same volume as the solvent, *i.e.* one gramme molecule of solute in 22·4 l. of solvent would have an osmotic pressure of one atmosphere (or 760 mm. Hg). We see that the osmotic pressure of a solution, the measure of its attraction for water, represents a certain amount of potential energy, which can be utilised in an osmotic machine, such as that represented in the diagram, for the performance of work.

The amount of work done in this way can be calculated in exactly the same way as when we are dealing with a gas. To compress a gramme molecule of any perfect gas from volume V_1 to volume V_2 requires the expenditure of work represented by $W = RT \log_e \frac{V_1}{V_2}$. If we take the pressures, and compress from pressure p_1 to pressure p_2, the expression will be $W = RT \log_e \frac{p_2}{p_1}$. To convert from natural to ordinary logarithms, we must multiply this expression by 2·303. The numerical value of R depends on the units required, being 1·991 for gramme calories and 0 848 for kilogramme metres. T at 37° C. is 310° C. The equation then becomes:

$$W = \left.\begin{array}{l} 1421 \text{ gramme calories} \\ \text{or } 605{\cdot}5 \text{ kilogramme metres} \end{array}\right\} \times \log \frac{p_2}{p_1}$$

To compress a fluid containing one gramme molecule of solute from osmotic pressure π_1 until it is concentrated to an osmotic pressure π_2, would require 605 5 $\times \log \frac{\pi_2}{\pi_1}$ kilogramme metres.

It must be remembered that this expression gives the work done in concentrating a solution (whatever method of concentration be applied, *e.g.* evaporation) at a constant temperature.

THE MEASUREMENT OF OSMOTIC PRESSURE. By a method differing but little from the one just sketched out, it is possible directly to measure the osmotic pressure of aqueous solutions. A semi-permeable membrane is provided when certain substances are precipitated in films, and one of these is copper ferrocyanide deposited within the interstices of a clay cell. Strengthened in this way, it is able to afford a resistance to pressure, and therefore to permit of the contained fluid reaching its full osmotic pressure. For this purpose a porous pot carefully cleansed and containing a solution of sugar mixed with a little copper sulphate is dipped into a weak solution of potassium ferrocyanide. A semi-permeable membrane of copper ferrocyanide is thus produced in the pores of the filter. The pot is then filled with the required solution, fitted with a cork provided with a closed mercurial manometer, and is immersed in distilled water, which passes into the cell until the pressure within the latter is equal to the osmotic pressure of the dissolved substances. By this means Pfeffer obtained the results given in the table below with a 1 per cent. solution of cane sugar at different temperatures.

By Avogadro's hypothesis, equal volumes of gases at the same pressure contain equal numbers of molecules. The figures obtained by Pfeffer show that the same laws apply to the osmotic pressure of substances in solution as to the pressure of gases in their free state. It is therefore possible to reckon the osmotic pressure which would be exerted by 1 per cent. sugar in solution at a given temperature.

Temp. °C.	Pressure in atmospheres.	
	Observed.	Calculated.
	Atm	Atm.
6·8	0·664	0·665
13·7	0·691	0 681
22·0	0·721	0·701
32·0	0·716	0·725
36·0	0·746	0·735

This calculation is carried out as follows: A gramme molecule of any gas at 0° C. and 760 mm. Hg has a volume of 22·4 litres, therefore 342 grammes of cane sugar (one mole), if it could be converted into a gas at 0° C. and 760 mm. Hg, would have a volume of 22·4 litres. In Pfeffer's experiment one gramme of sugar was dissolved in 100 grammes of water, making a total volume at 0° C. of 100·6 c.c. The osmotic pressure of the sugar molecules in this solution will therefore amount to $\frac{22400}{342 \times 100{\cdot}6} = 0{\cdot}651$ atmosphere. At a temperature of 6·8° the pressure would be 0·665 atmosphere, as against the observed 0·664 atmosphere.

Pfeffer's method is difficult to carry out and is not applicable to all dissolved substances, since the cupric ferrocyanide membrane is permeable for many substances, such as potassium nitrate or hydrochloric acid. Other indirect methods have therefore been applied to the comparison of the osmotic pressures of different solutions.

Determination of Osmotic Pressure by Plasmolysis. Solutions which have the same osmotic pressure are spoken of as isosmotic. The method of plasmolysis, which we owe to the botanist De Vries, consists essentially in the comparison of the osmotic pressure of solutions with that of the cell sap of certain plant cells, and depends on the fact that the 'primordial utricle,' the layer of protoplasm enclosing the cell sap, while freely permeable to water, is impermeable to a large number of salts and other crystalloids, such as sugar. It is, therefore, so far as concerns these substances, 'semi-permeable.' The cells which have been most used for this purpose are the cuticular cells on the midrib of the lower surface of the leaves of *tradescantia discolor*. If some of these cells are brought into a concentrated salt solution, which is 'hypertonic' as compared with the cell sap, water passes out of the cell into the salt solution, until the contents of the cell attain a molecular concentration equal to that of the surrounding medium. The protoplasmic layer therefore shrinks, leaving a space between it and the cell wall (Fig. 8, p. 19). If the outer solution has a smaller molecular concentration than the cell sap, water passes into the cell and causes here a rise of pressure which simply presses the protoplasm still more closely against the cell wall. If we determine the concentration of the salt solution at which the shrinkage of the protoplasm, the *plasmolysis*, just occurs, and another smaller concentration at which plasmolysis is absent, we know that the concentration of the cell sap lies between those of the two salt solutions. Thus, if plasmolysis occurs in a solution containing 0·6 per cent. sodium chloride and is absent in a solution containing 0·59 per cent. of the same salt, the concentration of the cell sap must be about equivalent to a 0·595 per cent. NaCl solution. Solutions of different salts, in which plasmolysis just occurs, must also be isotonic with one another. Thus a 1·01 per cent. solution of KNO_3 is found to be isotonic with a 0·58 per cent. NaCl solution.

Determination by Hamburger's Blood Corpuscle Method. The limiting external layer of red blood corpuscles resembles the primordial utricle of plant cells in being impermeable to a number of dissolved substances. If red corpuscles be placed in a solution of smaller concentration than their contents, they will swell up and, since they have no supporting cell wall, the increase in size may go on until the corpuscles burst, and their contained red colouring matter, hæmoglobin, passes into solution in the surrounding fluid. If the corpuscles be then allowed to settle or be centrifuged, the fact that hæmolysis has occurred is shown by the red colour of the clear supernatant fluid. In order to determine the osmotic pressure of a solution, say of sugar or of sodium chloride, these are added in various dilutions to blood corpuscles until we get solutions

in which hæmolysis just occurs. These are called *isotonic* solutions. As an example of this method may be adduced the following results:

	Lowest concentration of the solution in which the blood corpuscles do not lose hæmoglobin.	Concentration of the solution in which the blood corpuscles begin to lose hæmoglobin.	Mean concentration.
	Per cent.	Per cent.	Per cent.
Potassium nitrate . .	1·04	0·96	1·00
Sodium chloride . .	0·60	0·56	0·585
Cane sugar . . .	6·29	5·63	5·96
Potassium iodide . .	1·71	1·57	1·64
Sodium iodide . .	1·54	1·47	1·505
Potassium bromide . .	1·22	1·13	1·17

Isotonic is not necessarily identical with isosmotic. Tonicity has reference to the action of the solution with regard to some cell membrane, and therefore implies the impermeability of the membrane to the substance or substances in solution. Thus equimolecular solutions of urea and of glucose would be isosmotic, yet, tested by the blood corpuscle method, the solution of urea would be found to be isotonic with distilled water, since the outer layer of a blood corpuscle is freely permeable to urea, but not to sugar. Urea is therefore unable to exert any osmotic pressure in relation to blood corpuscles.

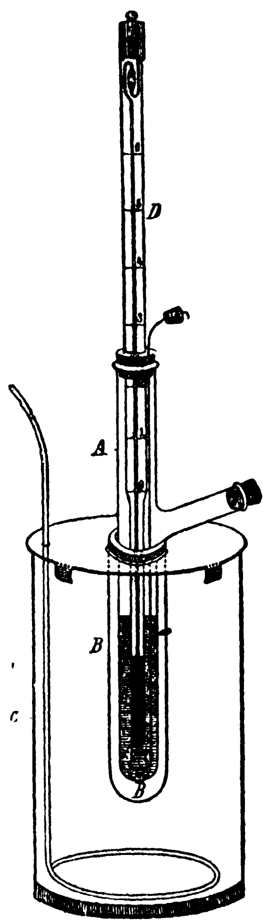

FIG. 16. Beckmann's Apparatus for Determination of Freezing Point.

INDIRECT METHODS OF MEASURING OSMOTIC PRESSURE. Equimolecular solutions (of non-electrolytes) have the same osmotic pressures. Since the osmotic pressure of such a solution is dependent on the number of molecules of solute in unit volume, any method which will tell us this will also enable us to determine the osmotic pressure. Other properties of solutions which are functions of the number of molecules present, are vapour tension, boiling point, freezing point. The presence of a substance in solution in water diminishes its vapour tension at any given temperature, raises its boiling point, and depresses its freezing point; and the extent of the deviation is proportional to the number of dissolved molecules present. The determination of the rise of boiling point is of very little value in physiology.

The classical method is the measurement of the *depression of freezing point* (generally represented by Δ).

The determination is carried out with a thermometer reading to $\frac{1}{100}$°C. (Fig. 16). The freezing point of a decimolar solution of a non-electrolyte is $-0{\cdot}186°$ C., and that of a molar solution (*i.e.*, containing one gramme molecule per litre, in the case of glucose 180 grammes), would have a Δ of 1·86° C. Since this has, as already explained, an osmotic pressure of 22·4 atmospheres, it is evident that the depression of freezing point can be converted directly into osmotic pressure by multiplying the depression by $\frac{22{\cdot}4}{1{\cdot}86} = 12{\cdot}04$. Thus a 1 per cent. solution of sodium chloride with $\Delta = 0{\cdot}61$ will have an osmotic pressure of $0{\cdot}61 \times 12{\cdot}04 = 7{\cdot}34$ atmospheres, while a 1 per cent. cane sugar solution with a $\Delta = {\cdot}0546$ will have an osmotic pressure of 0·675 atmosphere. This method has the advantage that the fluids are in most cases

not altered by freezing. For colloid solutions, since the osmotic pressures are small, the values of Δ are very small.

A simple and accurate method was introduced by A. V. Hill *; it depends on the determination of the *depression of vapour pressure* by the dissolved substances, by observation of the differences in temperature produced by evaporation. Since it involves the use of thermopiles it is extremely delicate. The method makes use of the fact that if two aqueous solutions of different osmotic (*i.e.* vapour) pressures are placed in a moist atmosphere, water will be evaporated faster (or condensed more slowly) at the surface of the solution having the lower than from the one with the higher osmotic pressure, and hence the solution with the lower osmotic pressure will become cooler than the other. If the two solutions are in contact with the two faces of a thermopile connected with a galvanometer, the difference in temperature produces a deflection proportional to the differences in osmotic pressure. If the osmotic pressure of one of the two solutions is known, that of the other can be deduced. In practice a piece of filter paper is wetted with each of the two solutions, *e.g.* blood on one and 0 92 per cent. NaCl on the other, and these are applied one to each face of the thermopile. This is then placed in a moist chamber kept damp by the known solution and at constant temperature, and connected with the galvanometer. Readings are taken after half an hour. Example: Blood and 0 92 per cent. NaCl. Galvanometer reading = 19 8 mm. Calibration showed

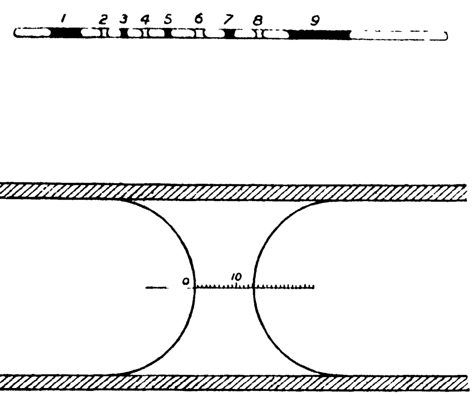

FIG. 17. Diagram to illustrate Barger's Method of Determining Osmotic Pressure. The upper figure shows the capillary tube with nine alternate drops of cane sugar and the substance under investigation.

that 1 mm. deflection = ·001120 grms. NaCl per cent. Hence difference of V.P. on two sides is = 19·8 × 001120 = ·0222 grms. per cent, *i.e.* the V.P. and O.P. of blood is equal to that of 0·92 + ·0222 = 0·9422 grms. NaCl per cent.

Barger suggested a simple method † for small quantities of fluids. Drops of the fluid are drawn up into a tube (1·5 mm. in diameter), so as to alternate with small drops of cane sugar solution of known content (Fig. 17). Water vapour will pass from the solution of which the vapour tension is the higher. By observing the edge of a drop under a microscope, it can be easily seen whether it has grown or diminished in size. If the edge of the drop remains stationary, it shows that the vapour tension and the osmotic pressure of the two fluids are equal. A series of trials is made with different strengths of sugar solution until this equality is established.

OSMOTIC PRESSURE OF ELECTROLYTES. The molecular weight of NaCl is 58·5; that of glucose is 180. We might therefore expect to find that a 0·58 per cent. solution of NaCl would be isotonic or isosmotic with a 1·8 per cent. solution of glucose. Actually it is isosmotic with a glucose solution of about 3·5 per cent., however the osmotic pressure be measured. This departure from the theoretical value of the

* HILL. *Proc. Roy. Soc. A.*, 1930, 127, 9.
† BARGER. *Trans. Chem. Soc.*, 1904, **85**, 286.

osmotic pressure, as deduced from the molecular weight, is connected with the fact that salt solutions, as well as solutions of acids and alkalies, are electrolytes, *i.e.* they ionise. Pure water hardly conducts a current at all, and its conducting power is little altered by the addition of sugar, but the smallest trace of salt, acid or alkali added to distilled water enormously improves its conducting power. Arrhenius showed that the conducting power of electrolytes depends on the fact that their molecules, in the act of solution in water, undergo dissociation into ions which increases with the dilution of the solution. Since each ion as regards osmotic pressure and the related properties acts as a whole molecule, the osmotic pressure of a *dilute* solution of NaCl is double that of an equimolecular solution of glucose. If the solution is one of sodium sulphate the osmotic pressure is increased three times, since this salt dissociates into two Na ions and one SO_4 ion.

Every substance in solution evidently possesses a certain amount of potential energy in the form of osmotic pressure. This pressure is independent of the nature of the substance dissolved and is determined merely by its molecular concentration and degree of ionic dissociation. It can be used as a force for the attraction of water or, by the use of appropriate mechanisms, for the performance of mechanical work or, as will be seen later, for the production of electrical differences of potential.

"NORMAL" PHYSIOLOGICAL SOLUTIONS. Using blood corpuscles, we may find a concentration, *e g.* of sugar at which the corpuscles will neither increase nor diminish in bulk. This concentration varies according to the animal from which the corpuscles are derived. Instead of sugar we can use sodium chloride. It is then found that a 0·65 per cent. NaCl solution is isotonic with frogs' blood corpuscles, and that a 0·9 per cent. NaCl solution is isotonic with mammalian red corpuscles. Since the osmotic concentration is identical in all the cells of the body and is the same as that of the blood, 0·65 per cent. and 0·9 per cent. NaCl solutions have been given the name of '*normal saline*,' and are used for maintaining tissues, respectively of frog or mammal, moist when they are under experiment.

Since the limiting external layer of most cells seems to resemble blood corpuscles in being impermeable to sugar and neutral salts, it might be thought that it was a matter of indifference what salts were used for making normal saline. This is not the case. It was shown by Ringer (Ch. XXXII) that to sodium chloride there must be added traces of calcium and potassium salts in order to obtain a saline solution suitable for keeping the heart alive and beating.

The function of a cell is thus dependent, not only on the nature of its contents and on the osmotic pressure of the surrounding fluid, but also on the nature of the ions which are in contact with its external surface. It should be noted that it is a matter of indifference what salts of calcium or potassium are used, since it is the metallic ions themselves which are responsible for the effect. When we wish to keep a tissue alive, immersed in some solution, we employ a 'Ringer's fluid' in which sodium, potassium and calcium ions are present in the proper amounts. It is customary to add also some sodium bicarbonate and sometimes sodium phosphate, so as to imitate the reaction of the normal body fluids and to provide a means of neutralising small amounts of acid which may be produced during the activity of the tissue under investigation. The accompanying table shows the composition of some commonly employed physiological saline solutions.

PHYSIOLOGICAL SALINE SOLUTIONS.

	Ringer (Frog).	Ringer-Locke	Ringer-Tyrode.	Ringer-Dale.	Buffered Ringer [C.L.E.].
Water	100·0	100·0	100·0	100·0	100·0
NaCl	0·65	0·9	0·8	0·9	0·85
KCl	0·014	0·042	0·02	0·042	0·042
$CaCl_2$	0·012	0·024	0·02	0·024	0·024
$NaHCO_3$	0·02	0·01–·03	0·1	0·05	—
NaH_2PO_4	0·001	—	0·005	—	—
$MgCl_2$	—	—	0·01	0·0005	—
Glucose	[0·2]	[0·1–0·25]	0·1	0·05	0·1
Na_2HPO_4	—	—	—	—	0·06
$\frac{M}{1}$ H_3PO_4	—	—	—	—	0·02–0·06 c.c.

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NEUTRALITY AND REACTION

Most of the tissues in the human body contain about 80 per cent. of water. In every reaction taking place in the organism water plays an essential part. On dissociation the water molecule forms oppositely charged hydrogen and hydroxyl ions, and it is calculated that 1 litre at 22° C. contains 10^{-7} gramme ions of hydrogen, and of course the same number of hydroxyl ions. The temperature coefficient of the dissociation of water is fairly high, so that at 18° C. the hydrogen ion concentration in pure water is only $0{\cdot}78 \times 10^{-7}$, while at 37° C. it is $1{\cdot}76 \times 10^{-7}$.

Acidity is due to the presence of hydrogen ions, while alkalinity is due to the hydroxyl ions. When they are equal in concentration the reaction is said to be neutral. When a strong acid such as hydrochloric acid is added to a strong base such as NaOH, the hydrogen and OH ions combine to form molecules of water, leaving behind only Na and Cl ions. The actual combination which takes place is thus the same whatever strong acid and strong base are used, namely, a combination of H and OH ions. In a solution containing H and OH ions, the active mass in any reaction which may take place is equal to the product of the two concentrations H $\times$ OH. In a watery solution this is constant for any given temperature and amounts at 22° C. to 10^{-14}, increasing with the temperature; (at 37° it is $3{\cdot}1 \times 10^{-14}$). Thus the more H ions are present in a solution the fewer will be the OH ions, and the shift is considerable, since it is the product of the two and not the sum which remains constant.

The chemical reactions taking place in any solution in which H ions are concerned, or the behaviour of a living cell bathed by the solution, will be determined, not by the total amount of alkali requisite to neutralise the acid, but by the actual concentration or activity of the H ions in the solution. It has therefore become of extreme importance to be able to express the reaction of the body fluids in terms of H ion concentration, denoted by cH, or of H ion activity *ca*H.

Owing to the inconvenience of expressions such as $1{\cdot}05 \times 10^{-7}$, $1{\cdot}3 \times 10^{-6}$, &c., it has become customary to use, not the cH figure directly, but the negative exponent

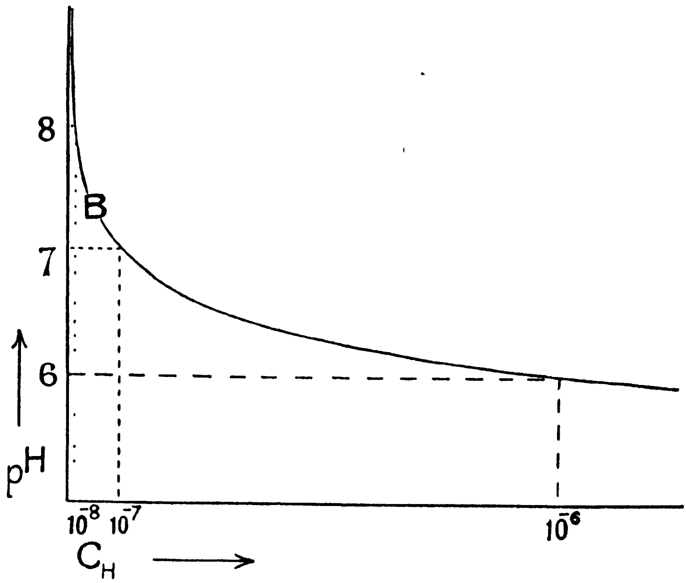

FIG. 18. Diagram to show the Relation between the two Methods of Registering Hydrogen Ion Concentration. (D. T. HARRIS)

of 10 as a positive number, calling it the hydrogen ion exponent or pH. Thus 5×10^{-6} is the same as $10^{-5{\cdot}3}$, and a solution having this concentration in H ions would be described as having a pH of 5·3. It must be remembered that the pH falls as the acidity increases. Moreover the change expressed by a unit difference in pH is much greater than that expressed by a unit difference in cH. Thus, while it is easy to see that the cH of 4×10^{-6} is double that of 2×10^{-6}, it is not at once obvious that a pH of 5·398 signifies an acidity which is double that of 5·699. The relation between the values of cH and of pH between cH 10^{-8} and cH10^{-6} is shown in Fig 18.

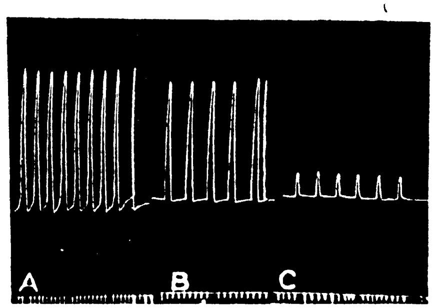

FIG. 19. Effect of Acid on the Isolated Heart of the Frog. (CLARK.)
A. Perfused with Ringer's solution with H′ ion concentration of pH 7·7.
B. After perfusion for 20 minutes with faintly acid Ringer's solution, H′ ion concentration, pH 6·5.
C. After perfusion of the acid solution for 80 minutes.

Since H and OH ions are involved in all the chemical processes which go on in the living cell, it is not surprising that the cell functions are profoundly modified by differences in reaction. In Fig. 19 is shown the effect of a change from $10^{-7{\cdot}7}$ to one of $10^{-6{\cdot}5}$ on the isolated heart of the frog. This change would be produced by the addition of 0·036 milligrammes of HCl to 1 litre of distilled water. A slightly higher degree of acidity, *i.e.* one of 10^{-6}, would kill the heart altogether.

'BUFFER' SYSTEMS. The changes of reaction which would be

caused by production of minute quantities of acid or alkali in the various chemical processes continually taking place in the cell are minimised by the presence of certain salts in the protoplasm and fluids of the body, in which the cells are bathed. These salts function as 'buffers' in taking up acid or alkali without a corresponding change in the reaction of the medium. The two principal are the bicarbonate system and the phosphate system, the former of chief inportance in the fluids of the body, the latter in the cells.

The phosphate system is made up of the two phosphates NaH_2PO_4 and Na_2HPO_4. In a watery solution of NaH_2PO_4 there are present, besides the $H^{\cdot}$ and OH' ions from the water, $Na^{\cdot}$, H_2PO_4', H , and HPO_4'' ions from the phosphate. The $H^{\cdot}$ ions are therefore present in greater quantity than in pure water, and the reaction is slightly acid. In a solution of Na_2HPO_4, the ions resulting from dissociation of the phosphate are $Na^{\cdot}$, $Na^{\cdot}$, and HPO_4''. The HPO_4'' ions combine with some of the $H^{\cdot}$ ions derived from the water to form H_2PO_4', leaving an excess of OH' ions, so that the reaction is slightly alkaline. Since it will require an equivalent weight of NaOH to convert 1 gramme molecule of NaH_2PO_4 into Na_2HPO_4, and 1 gramme molecule of HCl to effect the reverse change, a mixture of the two phosphates will absorb relatively large quantities of acid or base with but slight changes in the resulting reaction.

The acidity (*i.e.* the hydrogen ion concentration) of a mixture of the two phosphates will be proportional to $\frac{NaH_2PO_4}{Na_2HPO_4}$. When this proportion equals $\frac{1}{2{\cdot}5}$ the reaction will be neutral.

A still more perfect buffer system is afforded by $NaHCO_3$, when the solution is maintained in equilibrium with an atmosphere containing a constant proportion of CO_2. The solution will contain the following ions produced by dissociation.

$$
\begin{array}{lcl}
NaHCO_3 & \rightleftarrows & Na^{\cdot} + HCO_3'. \\
H_2CO_3 & \rightleftarrows & H^{\cdot} + HCO_3'. \\
H_2O & \rightleftarrows & H^{\cdot} + OH'. \\
HCO_3' + H_2O & \rightleftarrows & H_2CO_3 + OH'.
\end{array}
$$

H_2CO_3 is a very weak acid, so that only few hydrogen ions are produced. If an acid be added to the solution, a certain amount of the $NaHCO_3$ is decomposed with the formation of CO_2 and a neutral salt. But the CO_2 escapes from the solution, so that the only result is a diminished concentration of the $NaHCO_3$.

The cH of the solution will be proportional to $\frac{H_2CO_3}{NaHCO_3}$, and the reaction will be neutral when $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{3{\cdot}75}$.

The salts of any weak acids (*e.g.* boric, acetic, &c.) or weak bases, *e.g.* ammonia, may act as buffers, and we may have complex systems in which these substances are present. Such buffer mixtures are of great value in physiological work because, by mixing two such substances in known proportions, solutions of definite hydrogen ion concentration may be prepared; such solutions only slowly change their H-ion concentrations when acids or bases are added to them. Examples of such buffer mixtures are given below.

Range of Buffer Mixtures

Mixtures of	Range of pH obtained.
Glycine and HCl	1·15 — 3·68
Acid K. Phthalate and HCl . .	2·2 — 3·8
Acid K. Phthalate and NaOH . .	4·0 — 6·2
Acetic acid and sodium acetate .	3·24 — 6·55
$K_2HPO_4 + KH_2PO_4$	5·19 — 8·21
Boric acid, HCl and NaOH . .	7·8 — 10·0
Glycine + NaOH	8·58 — 12·97

Among other important buffers present in cells the proteins must be mentioned. Like the amino-acids, *e.g.* glycine shown above as a laboratory buffer, the proteins can function as either weak bases or weak acids and so absorb considerable amounts of either acid or alkali, so helping to stabilise the H-ion concentration of cells.

THE MEASUREMENT OF HYDROGEN ION CONCENTRATION

The Hydrogen Electrode. When a metal, say copper, is immersed in a solution of one of its own salts, such as copper sulphate, the copper has a tendency

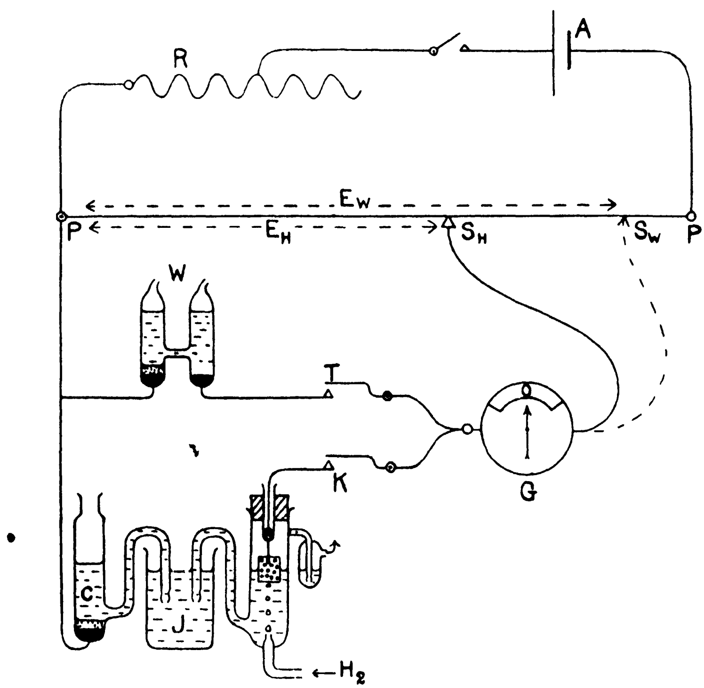

Fig. 20. Arrangement of Apparatus for Determination of H Ion Concentration, cH. (D. T. Harris.)

to give off Cu ions into the solution: as these carry a positive charge, their loss will leave the metal negatively charged. On the other hand, the Cu ions already in the solution will tend to pass to the metal and to impart to it a positive charge. There will thus be a difference of potential between metal and solution, the extent and sign of the difference depending on the relative strengths of the two forces concerned, namely, the electrolytic solution pressure of the copper and the osmotic pressure of the Cu ions in the copper sulphate solution. A hydrogen electrode

depending on similar principles may be made from platinum saturated with hydrogen. With such an electrode the forces concerned in determining the difference of potential between the metal and the solution in which it is immersed, will be the electrolytic solution pressure and the activity of the hydrogen ions in the solution. By measuring the potential difference between such an electrode and a solution, we can estimate the concentration of hydrogen ions in the solution. The arrangement for this purpose is shown in the accompanying diagram (Fig. 20).

A calomel electrode C is connected up by a junction vessel J, filled with saturated KCl solution, to a hydrogen electrode immersed in the solution of which the hydrogen ion concentration is to be determined. The hydrogen electrode consists of a piece of platinum (or gold) on which platinum black has been deposited. This is saturated with pure hydrogen gas, which is allowed to bubble slowly through the fluid by the tube H_2. The E.M.F., E at 38° is measured, and the pH found from the equation :—

$$pH = \frac{E - 0.235}{0.062}$$

0·235 volt is the E.M.F. set up at 38° C. between a saturated KCl—calomel electrode and a hydrogen electrode in a normal solution of hydrogen ions. The E.M.F. actually observed is measured by comparing it with that of a standard (Weston) cell (W) as shown in the Figure, where R is a resistance, A an accumulator, EW a calibrated potentiometer, and G a galvanometer. By means of the resistance R, the current through the potentiometer wire is balanced against the standard cell, so that no current flows through the galvanometer when the key T is closed. By means of the rider S_H a fraction of this potential is balanced against the E.M.F. of the hydrogen-calomel electrode battery until no current flows through the galvanometer on closing K. From the length of wire between P and the rider, we can read off at once the E.M.F. of the hydrogen-calomel battery, and thereby determine the hydrogen ion concentration in the fluid surrounding the hydrogen electrode.

The Glass Electrode. A method can be used which depends on the fact that a potential difference is set up between two solutions of different hydrogen ion concentrations, when these are separated by a very thin glass membrane. If the hydrogen ion concentration of one of the solutions is known, that of the other can be calculated from the difference of electrical potential. The method requires a special technique,* but has certain advantages, one of which is that it is not necessary to de-oxygenate the solution.

Indicators. In most cases the estimation can be made rapidly by means of *indicators*. These are dyes which change colour at definite values of hydrogen ion concentration.

COMMON INDICATORS FOR pH DETERMINATIONS.

Indicator.	Range, pH.	Acid Colour.	Alkaline Colour.
Methyl violet	0·1– 3·2	Yellow.	Violet.
Thymol blue (acid range)	1·2– 2·7	Red	Pale yellow.
Bromphenol blue	3·0– 4·6	Yellow.	Blue.
Methyl orange	3·2– 4·6	Red	Yellow.
Congo red.	3·5– 4·7	Blue	Red.
Bromcresol green	4·0– 5·6	Yellow.	Blue green.
Methyl red	4·3– 6·3	Red	Yellow.
Neutral red	6·5– 8·4	Red (blue in strong acid solutions).	Yellow.
Bromcresol purple	5·2– 6·8	Yellow.	Purple.
Phenol red	6·8– 8·4	Yellow.	Red.
Cresol red	7·2– 8·8	Reddish	Orange.
α-Naphthol-phthalein	7·6– 8·5	Yellow.	Blue.
Thymol blue (alkaline range)	8·0– 9·6	Pale yellow	Blue.
Phenol-phthalein	8·3–10·0	Colourless	Red.
Thymol-phthalein	9·3–10·5	Colourless	Blue.
Nitrothymolsulphonephthalein	9·2–11·5	Violet	Yellow.
Alizarin yellow	10·2–12·1	Yellow.	Orange.
Tropaeolin O	11·1–12·7	Yellow.	Orange.

* KERRIDGE. *Bioch. J.*, 1925, **19**, 611 ; *J. Sci. Insts.*, 1926, **3**, 404.

They are chiefly salts of either a very weak acid or a weak base, or the free acid or base itself, the change of colour being due to the electrolytic dissociation of the salt with the production of an ion which has a different colour from that of the undissociated acid or base. Some of the most useful indicators, and the range of their concentrations, are given in the accompanying table. In determining the hydrogen ion concentration of fluids such as the blood, the reaction may be tested in the dialysate obtained by placing the blood in a thimble of collodion, which is immersed in a small quantity of 0 85 p.c. NaCl solution,* care being taken to avoid loss of carbon dioxide. The dialysate acquires the same reaction as the blood, which is determined by adding a suitable indicator (neutral red or phenol red) and matching against standard buffer solutions to which the same indicator has been added.

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SURFACE ENERGY

The forces at the surface of a fluid differ from those in its interior. At the surface, any molecule, instead of being subject to the attraction of the other molecules on all sides, is exposed only to those attractions which tend to draw it inwards, so that as a result the surface is always the least possible and is in a state of tension. This *surface tension* can be measured, and is expressed in dynes per centimetre. To stretch the surface requires the expenditure of energy, while work may be done if the surface is diminished. It is surface tension which causes water to rise in capillary tubes, and the height to which the water rises may be used as one means of measuring surface tension. If, as in the case of mercury, a fluid does not moisten the capillary tube, there is a depression of the meniscus. Any substance which lowers the surface tension of water will tend to spread over its surface until it is one molecule thick. This is the case with oil dropped on water, the spreading of the oil being due to the expansion of the surface of water at the part where its surface tension is diminished by contact with oil. Wherever we have two immiscible fluids there will be a surface tension, and in the same way there will be a surface tension between liquids and solids. The fact that protoplasm consists largely of colloids involves a heterogeneity of structure, and hence large areas of surfaces.

The surface tension of a liquid is altered by dissolved substances. Inorganic salts cause a slight rise of surface tension of a solution in contact with air. Most organic substances cause a diminution of surface tension. It was shown by Gibbs and Thomson that dissolved substances which lower surface tension tend to concentrate at the surface, on account of the fact that the free energy of the system is thereby lessened. In many cases this concentration at the surface may be so marked that the substance forms a solid pellicule (p. 19).

When we are dealing with an interface between liquid and liquid or between liquid and solid, this concentration of solute or dissolved substance at the surface is equivalent to a deposition on the other substance with which the solution is in contact. It is responsible for many cases of concentration at surfaces, for which the name *adsorption* is used (*v.* p. 79). The marvellous power of adsorption of gases by charcoal is due to the very large surface presented by this substance. In the dyeing of stuffs and the staining of sections adsorption is the chief factor involved. On chemically active solutes this process of adsorption will have a two-fold effect: that part which is adsorbed at the surface is taken out of the main solution and loses, for instance, its osmotic effect. On the other hand, the concentration of two reactive substances in the same surface may bring about their interaction, so that chemical changes are furthered which without this concentration would have gone on much more slowly.

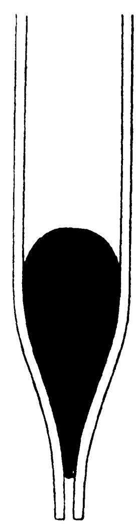

FIG. 21.

Many surfaces are the seat of an electric charge. Most insoluble substances such as glass, paper, or wool, in water are negatively charged. This charge affects the type of adsorption at the surface of such particles, and will tend to favour the concentration of electro-positive as against electro-negative molecules at the interface. A charge on a surface implies a diminution of the surface tension.

* DALE and LOVATT EVANS. *J. Physiol.*, 1920, **54**, 167.

The construction of the capillary electrometer depends on this fact. When mercury is in contact with dilute acid, it takes a positive charge and the state of stress at the surface of contact between the mercury and the negatively charged fluid diminishes the surface tension of the mercury. If mercury be in a tube drawn out to a capillary, it will run down the capillary until the surface tension tending to pull the mercury into a spherical globe is just equal to the force of gravity tending to make the mercury run out through the end of the capillary (Fig. 21). If the capillary be filled with sulphuric acid, the mercury will descend to a lower level in the capillary owing to the diminution of its surface tension. If now the acid and the mercury be connected with a source of current so as to charge the mercury negatively, the effect will be to diminish the charge previously on the mercury. The surface tension is increased, and the mercury withdraws itself from the point of the capillary. If, however, the mercury be connected with the positive pole, its charge will be increased and its surface tension correspondingly diminished, so that the meniscus will move towards the point of the capillary. The movement of the meniscus may thus be used, to show the direction and amount of any moderate electric potential (see also p. 168).

Since electrolytes may augment or diminish the charge at a surface, we see how important may be the constituents of a solution to the living cells with which it has to come in contact.

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THE FREE ENERGY OF CHEMICAL PROCESSES

By "free energy" is meant energy at high potential capable of performing work. All the energies of the animal body are derived ultimately from the processes of oxidation occurring by slow degrees in the cells of the body. For many practical purposes the heat of oxidation of one of the ordinary foodstuffs does not differ essentially from the free energy of that change. This is not necessarily so, however, because the change in free energy is only equal to the heat of reaction under certain conditions. When the free energy of a cell is degenerated into heat energy, it is wasted.

We know that in our machines only a small proportion of heat energy can be utilised for work, and in an isothermal system none at all. Within the minute space of a cell there is no possibility of any significant differences of temperature : the whole cell cools or warms together.

But the life of a cell or of an animal, composed of myriads of these minute isothermal units, involves many processes which mean external or internal work. For these purposes free energy is required, and this must be derived from chemical changes occurring in the living cell independently of the production of heat. In any isothermal change the free energy is the maximum external work which could be done under the best imaginable conditions ; the free energy of a change is independent of the path of change, as is the total energy—the heat production , the free energy may, in general, be greater than, equal to, or less than, the heat production. It involves a system, *i.e.* it is organised energy, whereas heat is unorganised energy, only convertible into work under special conditions.

In the circumstances existing in the living cell every reaction or change in the system will tend to diminish the free energy, the loss being equal to the maximum external work which the system could have produced in the change. As a matter of fact, the system is never perfect, nor is the maximum work attained, the difference appearing as heat and being wasted, except when it can be utilised for maintaining a stable body temperature, at a level above that of the surrounding medium.

It is important to note that the second law of thermodynamics, which affirms the diminution of free energy in any isothermal change, applies to the whole (supposed isolated) system, even when this is a cell of microscopic dimensions, and not to any individual constituent of the system. Thus we find that in the body there may be changes which raise the chemical potential of a substance, *i.e.* convert it into another body having more potential energy, provided the reaction is coupled with one involving a diminution of potential. Thus it is probable that the recovery process in muscle, where a small fraction of the lactic acid formed in the previous contraction is oxidised with the production of heat, while the greater part of the lactic acid is reconverted into glycogen, belongs to this class of coupled reactions.

CHAPTER VI

THE PROPERTIES OF COLLOIDS

THE important part played by colloids in the processes of life renders it necessary to discuss their properties in some detail.

The term colloid, from κόλλη, glue, was first introduced in 1861 by Thomas Graham, Professor of Chemistry at University College. Graham divided all substances into two classes, viz. *crystalloids*, including such substances as salt, sugar, urea, which could be crystallised with ease, diffused rapidly through water and were capable of diffusing through dead animal membranes; and *colloids*, which included substances such as gelatin, gum, egg-albumen, starch and dextrin, were non-crystallisable, formed gummy masses when their solutions were evaporated to dryness, diffused with extreme slowness through water and would not pass through animal membranes. The process of *dialysis* was therefore introduced by Graham for the separation of crystalloids from colloids. Although the broad distinction drawn by Graham between colloids and crystalloids still holds good, some of the criteria by which he distinguished the two classes are no longer strictly applicable. For instance, it has been shown that many typical colloids, such as proteins, can be obtained in a crystalline form. On the other hand all gradations exist between substances, such as egg-albumen, which are practically indiffusible, and those, such as common salt, which are very diffusible. Colloids exist under two conditions:

(1) In a state of solution or pseudo-solution, in which they form *sols*, and are distinguished as hydrosols when the solvent is water; and

(2) In a solid state, in which a relatively small amount of the colloid sets with a large amount of a fluid, such as water, to form a jelly. This solid form, *e.g.* gelatine, is known as a *gel*. Such a watery jelly is known as a *hydrogel*.

A number of these colloidal substances can be shown on purely chemical grounds to consist of monstrous molecules. Thus the molecular weight of hæmoglobin is at least 64,000, and one must ascribe similar high molecular weights to such substances as egg-albumin and globulin. Still greater must be the molecular size of such substances as the cell proteins, which may form a gigantic complex, to which it would probably not be an exaggeration to ascribe a molecular weight of over 100,000. Chemical complexity is not, however, a necessary condition of the colloidal state, as is shown by the existence of colloidal silica, and, indeed, of colloidal solutions of practically any given substance, including many elements.

All so-called colloidal solutions are in reality suspensions of particles, the so-called *disperse phase*, in a fluid or *continuous* phase. The fluid may be any liquid or solution whatever. Thus, milk is a suspension of oil droplets in a watery phase, and butter is a colloid consisting of water suspended in a continuous phase of oil. The conversion of milk into butter is an example of phase reversal (*v.* p. 21).

Of special interest are the hydrosols of the metals. Faraday long ago pointed out that, on treating a weak solution of gold chloride with phosphorus, it underwent

reduction with the formation of metallic gold. The gold was not precipitated, but remained in suspension or pseudo-solution, giving a deep red * or a blue liquid, according to the conditions under which the reaction was effected. This solution was homogeneous in that it could be filtered without change and could be kept for months without deposition of the gold. The latter was, however, thrown down on addition of mere traces of impurity, though greater stability could be conferred on the solution by adding to it a little gelatin. In 1899 Bredig showed how similar hydrosols might be prepared from a number of different metals, viz. by the passage of a small arc or electric sparks between metallic terminals submerged in distilled water. If for example the terminals be of platinum, the passage of the current is seen to be accompanied by the giving off of brown clouds, which spread into the surrounding fluid. These clouds consist of particles of platinum of all sizes; the smaller—which are ultra-microscopic in size, *i.e.* from 5 m.μ to 40 m.μ—remain in suspension, and we obtain a brown fluid which can be filtered through paper or even through a Berkefeld filter without losing its colour. It may be kept for months without any deposit taking place. The addition of minute traces of electrolytes precipitates the platinum particles, leaving a colourless fluid.

Colloidal solutions or sols may be divided into two classes, *emulsoids* and *suspensoids*, according as they may be regarded as suspensions of liquid, or as suspensions of solid particles, in liquid.

Most protein solutions are emulsoids, while the metallic sols are suspensoids. Dilute egg-white is an emulsoid, but if it be boiled, although no visible precipitation is produced, the fine particles are coagulated and it behaves as a suspensoid.

Properties of Gels. The firm mass to which a solution of gelatin sets on cooling is a typical hydrogel. It is clear, hyaline, apparently structureless and elastic. It may be regarded as formed by the separation of the warm pseudo-solution of gelatin into two phases: first a solid phase, rich in gelatin and forming a tissue or meshwork in the interstices of which is embedded the second phase, consisting of a very weak solution of gelatin. In many cases the more fluid part of the gel is practically pure water. In a dry atmosphere the gel loses water and becomes shrivelled and dry, but in some cases, *e.g.* gelatin, it can resume its former size and characters on immersion in water. Other gels, such as silicic acid or ferric hydrate, lose the power of swelling up after drying. The change in them is therefore irreversible. A gel adheres to the last traces of water with extreme tenacity. In consequence of its structure, it presents an enormous extent of surface on which adsorption can take place. At this surface the vapour tension of fluids is diminished, as well as the osmotic pressure of dissolved substances.

Many colloids, even those such as starch or gelatin which are insoluble in cold water, absorb water and exhibit a phenomenon, viz. '*Quellung*' or imbibition, which in some cases it is impossible to distinguish from the process of solution. When soaked in aqueous solutions, the amount of water taken up varies inversely with the osmotic pressure of the solution. In many respects the insoluble emulsoid colloids when soaked in aqueous solutions, behave as though they were osmotic cells with boundaries capable of expansion. Indeed, we may regard them as such cells filled with colloid to which their surfaces are impermeable. When dry, colloids can exert a considerable force when caused to swell up by moistening.

The huge aggregates of molecules, which distinguish the colloidal state, form a system with considerable inertia, so that there is a tendency to the establishment of conditions of false equilibrium. Once a state is established, it is necessary to overstep widely the conditions of its formation in order to destroy it. Thus a 10 per cent. gelatin solution sets at 21° C. but does not

* Ruby glass is a colloidal 'solid' solution of gold in a mixture of silicates.

melt until warmed to 29·6° C. Solutions of agar in water set at about 35° C. but do not melt under 90° C. A gel of gelatin takes twenty-four hours after setting to attain a constant melting point. This phenomenon is called *hysteresis*.

On account of the extent of surface it is practically impossible to wash out the inorganic constituents from a gel. The diminution of the osmotic pressure of many dissolved substances at surfaces causes the concentration at the surface of the solid phase to be greater than that in the surrounding medium. Thus, if dry gelatin be immersed in a salt solution it will swell up, but the solution which it absorbs will be more concentrated than the solution in which it is immersed, so that the proportion of salt in the latter will be diminished. When, however, equilibrium is established between a gel and the surrounding fluid, it is found to present no appreciable resistance to the passage of dissolved crystalloids. Thus salt or sugar diffuses through a column of solid gelatin as if the latter were pure water. But gels are impermeable to other colloids in solution. This impermeability is made use of in the separation of crystalloids from colloids by dialysis, membranes used in this process being generally irreversible gels (*e.g.* vegetable parchment, animal membranes, collodion or cellophane). Other gels, such as tannate of gelatin or copper ferrocyanide, are not only impermeable to colloids, but also many crystalloid substances. These membranes can therefore be used for the determination of the osmotic pressure of crystalloids.

PROPERTIES OF HYDROSOLS. Hydrosols of substances such as dextrin or egg-albumin may be made of almost any concentration. If a solution of egg-albumin be concentrated at a low temperature, it becomes more and more viscous and finally solid. But there is no distinct point at which the fluid passes into the solid condition. Much discussion has arisen whether hydrosols are to be regarded as true solutions or as suspensions. The homogeneity or heterogeneity of a solution is clearly a matter of degree. In a true solution the molecules of the solute are equally diffused throughout the molecules of the solvent, and it is impossible, without the application of energy, to separate one from the other. Thus filtration or sedimentation leaves the composition of the solution unchanged. It is true that by the employment of the semi-permeable membrane, it is possible to separate solute from solvent, but in this case the force required to effect the filtration may be enormous. The measure of the force required is the osmotic pressure of the solution.

Osmotic Pressure.—Starling * showed that it is possible to determine the osmotic pressure of colloidal solutions directly, taking advantage of the fact that colloidal membranes, while permitting the passage of water and salts, are impermeable to colloids in solution. In this way it was found that blood serum, containing 7 to 8 per cent. of proteins, had an osmotic pressure of 25 to 30 mm. Hg, which would correspond to a molecular weight of ths protein, of about 30,000.

A convenient osmometer for this purpose is illustrated in Fig. 22. The membrane is of cellophane. The serum or other solution is placed in the brass capsule, and water on the upper surface of the membrane. The level of serum in the glass capillary is kept constant by applying counter-pressure from a manometer, to balance the osmotic pressure, and this counter pressure is read off when equilibrium is reached.†

The existence of an osmotic pressure in colloidal solutions was established by Moore and Roaf ‡ in the case of hæmoglobins, proteins and soaps, and by Bayliss in the

* STARLING. *J. Physiol.*, 1895, **19**, 323; 1899, **24**, 317.
† A simpler form is described by KEYS AND TAYLOR. *J. Biol. Chem.* 1935, **109**, 47
‡ MOORE and ROAF. *Bioch. J.*, 1907, **2**, 34.

case of colloidal dyes.* The osmotic pressure of hæmoglobin has been found by Adair † to correspond to a molecular weight of about 68,000, *i.e* about 4 times as great as the minimal molecular weight deduced from its combining power with oxygen. The osmotic pressure is often smaller than would be expected from the molecular weight of the substance, owing to molecular aggregation. Thus the aggregate of colloidal silica must be many, probably thousands of, times larger than the molecule as represented by H_2SiO_3. The osmotic pressure being proportional to the number of separate molecules or groups of molecules in a given volume of solution, to which a given membrane is impermeable, the larger the aggregate the smaller would be the osmotic pressure of the solution.

It is in consequence of the huge size of the molecular aggregates that colloidal solutions, such as starch or glycogen, display such a trivial osmotic pressure. We can divide colloidal solutions into two classes, viz. those which almost form true solutions and present a feeble osmotic pressure, and those

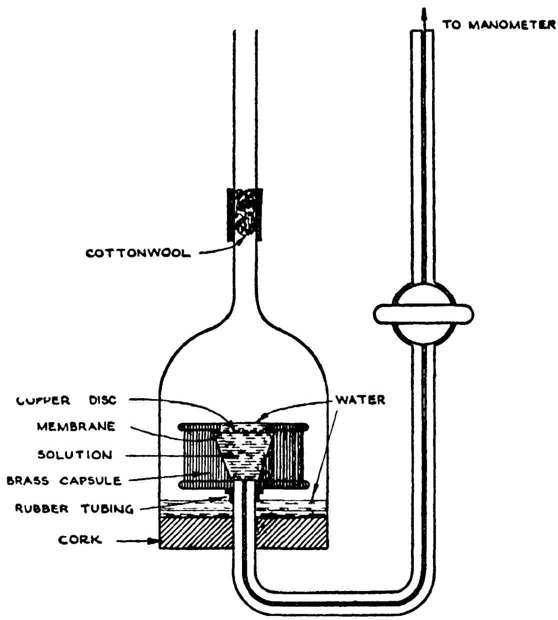

FIG. 22. Govaerts' Osmometer (Verney's modification). (KERRIDGE.)

which form suspensions and therefore exert no osmotic pressure. In inorganic colloids, such as arsenious sulphide, Picton and Linder have shown that all grades exist between true solutions and suspensions. With increasing aggregation of the molecules, the suspension becomes coarser and coarser until finally the sulphide precipitates.

Heterogeneity. We have every reason to believe that the very complex molecules forming the cell proteins may have molecular weights even greater than 30,000. When, however, we arrive at molecular weights of these dimensions, the disproportion between the size of the molecules and those of the water becomes so great that a homogeneous distribution of the two substances, solute and solvent, is no longer possible. The size of a molecule of water has been reckoned to be $0{\cdot}7 \times 10^{-8}$ mm. A molecule 10,000 times as large would have a diameter of $0{\cdot}7 \times 10^{-4}$ mm. $= 0{\cdot}07\,\mu$, a size just within the limits of microscopic vision. Long before molecules attained such a size they would no longer react according to the laws of solutes, but would possess the properties of matter in

* BAYLISS, W. M. *Proc. Roy. Soc.*, 1909, **81B**, 269; 1911, **84B**, 229.
† ADAIR. *Proc. Roy. Soc.*, 1925, **109A**, 292.

mass. They have a surface of measurable extent, and their relations to the molecules of solvent will be determined by the laws of adsorption at surfaces. As a matter of fact we find that such solutions present an amazing mixture of properties, some of which betray them as mechanical suspensions, while others show them to be capable of participating in chemical reactions such as those studied in the simpler compounds in solution usually dealt with by the chemist.

It has been found by Svedberg * that protein solutions and other colloids show partial sedimentation when submitted to extremely rapid centrifrugalisation, and he has calculated the apparent molecular weights of various proteins from their observed speeds of deposition. It would appear from his researches that most proteins have molecular weights of 17,600 or approximate multiples of this, *e.g.*:

Egg albumin	43,800
Hæmoglobin	69,000
Serum albumin	70,200
Serum globulin	167,000
H-Hæmocyanin	6,630,000

Optical Behaviour of Hydrosols. Nearly all colloidal solutions present what is known as the Faraday-Tyndall phenomenon. When a beam of light is passed through an optically homogeneous fluid, the course of the beam is invisible. A beam of sunlight falling into a dark room is rendered visible by impinging on and illuminating the dust particles in its course. Each of these particles, being illuminated, acts as a centre of dispersion of the light, so that the course of the beam is apparent to a person standing on one side of it. Tyndall showed that, if the particles were sufficiently minute, the light dispersed by them at right angles to the beam was polarised. This can be easily tested by looking at the beam through a Nicol's prism. The Tyndall phenomenon may be regarded as a test for the presence of ultra-microscopic particles, varying in size from 5 to 50 mμ. The phenomenon is perhaps too sensitive to be taken as a proof that a fluid presenting it is a suspension rather than a solution. It is shown, for instance, by solutions of many bodies of high molecular weight, such as raffinose (a trisaccharide) or the alkaloid brucine (Bayliss).

A particle having a diameter less than half the wave-length of light, *i.e.* about 300 λ or 0 3 μ, cannot be clearly distinguished under any power of the microscope. The fact that an ultra-microscopic particle may serve as a centre for dispersal of light may be used for rendering the *position* of such particles visible under the microscope. For this purpose a strong beam of light is passed in the plane of the stage of the microscope through a cell containing the hydrosol, which is then examined under a high power. On examining a dilute gold sol with this apparatus, we see a swarm of dancing points of light, "like gnats in the sunlight," which move rapidly in all directions, rendering it almost impossible to count their number in the field. The coarser particles present slight oscillations similar to those long known as the Brownian movement. The smallest demonstrable particles show a combined movement, consisting of a translatory movement, in which the particle passes rapidly across the field in one-sixth to one-eighth of a second, with one of oscillation of much shorter period. The representation of the course of such a particle is given in Fig. 23.

The size of the smallest particles revealed in this way may amount to 0 005 μ. Not all colloidal solutions show these particles in the ultra-microscope. In some cases this is due simply to the small size of the particles, and the addition of any substance, which causes aggregation and therefore increase in the size of the particles, will bring them into view. In others the absence of optical heterogeneity may be due to the coincidence of the refractive indices of the two phases of the hydrosol, or to the absence of any clear dividing surfaces between the two phases. In any case it is not the particle itself which is thus rendered visible, but only the light scattered from it.

ELECTRICAL PROPERTIES OF COLLOIDS

In the case of many hydrosols the ultra-microscopic particles of which they are composed carry an electric charge which, according to the nature

* SVEDBERG. *Transac. Faraday Soc.*, 1930, **26**, 740. *Nature*, 1937, **139**, 1051; *Proc. R. S.*, 1939, **127B**, 1.

of the solution, may be either positive or negative. On this account the particles move if placed in an electric field, and the direction of their movement reveals the nature of their charge. Thus colloidal ferric hydrate is electro-positive and travels to the cathode. The presence of a charge is not, however, a necessary condition for the stability of a colloidal solution. Thus the proteins of serum globulin in a neutral saline solution, or gelatin, present no drift when exposed to a strong electric field.* In certain colloids, the proteins for instance, the charge varies according to the conditions under which they are brought into solution. At a particular hydrogen ion concentration, differing for different proteins, and called the *isoelectric point*, the protein is uncharged and does not migrate in an electric field. When in solution on the acid side of their isoelectric points protein particles carry a positive charge, whereas on the alkaline side their charge is negative.

The charged condition of these particles must play a considerable part in keeping them asunder and therefore in preventing their aggregation and

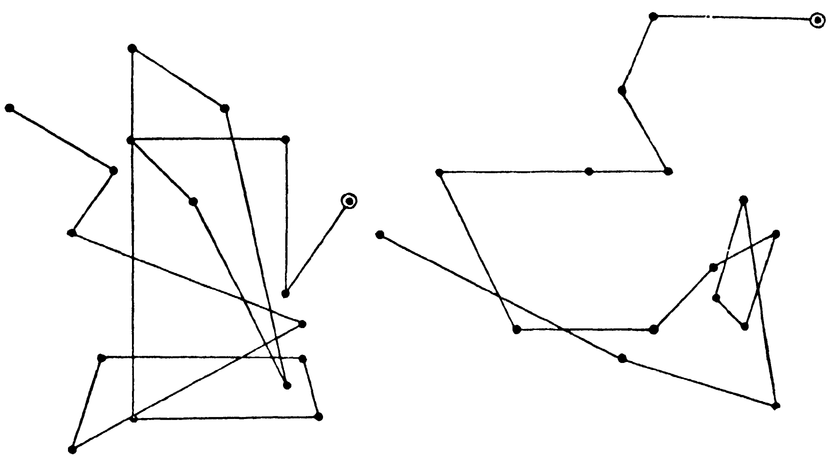

FIG. 23. Movements of two Particles of India-rubber Latex in Colloidal Solution, recorded by cinematograph and ultra-microscope. (HENRI.)

precipitation. This is shown by the fact that any agency which will tend to discharge them will often cause precipitation and coagulation. Among such agencies is the passage of a constant current and the coagulative effects of neutral salts. Thus, any of the colloids we have mentioned, ferric hydrate, silica, gold or boiled albumin, are thrown down by the addition of traces of neutral salts, and it is found that in this process they carry with them some of the ion with the opposite charge to that of the colloidal particle. Thus in the precipitation of the electro-positive ferric hydrate, the negative ion of the salt is the determining factor, the coagulative power increasing rapidly with the valency of the acid. On the other hand, in the precipitation of a gold sol, the electro-positive ion is the effective agent, and here again the coagulative effect is enormously increased by a rise in valency. This is shown by the fact that in the coagulation of gold, barium chloride with the divalent $Ba^{\cdot\cdot}$ is 7 times as powerful as K_2SO_4 containing the univalent $K^{\cdot}$; whereas, in the precipitation of the electro-positive ferric hydrate, K_2SO_4 with a divalent SO_4'' is 400 times as effective as $BaCl_2$.

* HARDY. *J. Physiol.*, 1905, 33, 251.

Coehn found that, when various non-conducting bodies are immersed in fluids of different dielectric constants, they assume a positive or negative charge according as their own dielectric constants are higher or lower than the fluid with which they are in contact. For instance, glass (5 to 6) is negative in water (80) or alcohol (26), whereas in turpentine (2·2) it is positive. In water, as Quincke has found, nearly all non-conducting parties take on a negative charge. Among these are cotton-wool, paper and silk. Particles of these in water, exposed to an electric field, move towards the anode.

THE HOFMEISTER SERIES. The action of ions on emulsoid colloids, *e.g.* proteins, is somewhat more complex than that described above, since both positive and negative ions exert an influence which is dependent on the nature of the ions. When gelatine is immersed in aqueous solutions, for example, its swelling is hastened by NaCl, NaBr, KCl, NH_4Cl and $NaNO_3$ and retarded by sodium sulphate, tartrate, citrate and acetate. It is possible to arrange certain ions in the order of their efficacies in affecting this and certain other properties of emulsoid colloids. This series, called the *Hofmeister series*, runs :—

$$\text{Sulphate, Tartrate} < \text{Acetate} < \text{Cl} < NO_3 < \text{I} < \text{SCN}$$

which exert a tendency, in the order shown, to precipitate positively charged protein, while the cations

$$\text{Li} > \text{Na} > \text{K} > NH_4$$

favour precipitation of positively charged (*i.e.* acidified) protein in the order shown. In alkaline solutions the order of each series is reversed. According to Loeb, if attention is given to hydrogen ion concentration, the series becomes altered and is then simply one of valencies. But it is probably important that the Hofmeister series also holds good for many aspects of the physiological effects of various ions in the tissues.

ADSORPTION. The charged condition of a colloidal particle makes it behave in an electric field in much the same way as a charged ion of an electrolyte, and this similarity extends also to its chemical behaviour, so that we can have a class of combinations formed resembling in many respects chemical compounds, but differing from them in the absence of definite quantitative relations between the reacting substances. This class of compounds of varying composition has been designated *adsorption complexes*.

The factors involved in the formation of adsorption combinations are :

(1) Extent of surface. In a colloidal solution this is enormous in proportion to the mass of substance in solution. Thus a 10 c.c. sphere with a surface of 22 sq. cm., if reduced to a fine powder consisting of spherules of 2·5 $m\mu$ in diameter, will have a surface of 20,000,000 sq. cm., *i.e.* nearly half an acre. At the whole of this surface adsorption may take place, involving the concentration of dissolved electrolytes, ions or gases.

(2) Chemical nature of particle, residual valencies, etc.

(3) Electric charge on the surface. The sign of this may be determined by the chemical nature of the colloid and its relation to the electrolytes in the surrounding medium.

The conditions which determine the formation of these adsorption compounds can be studied in their simplest form on the adsorption of dyestuffs by substances such as paper. If we take a series of solutions of a dye, such as Congo red, in progressively diminishing concentration, and place in each solution the same amount of filter-paper, we find that a part of the dye is taken up by the paper, and the proportion taken up is larger the more dilute the solution. This relation is in accordance with what Bayliss called the *law of adsorption*.* This is illustrated by the following Table :

* BAYLISS, W. M. *Bioch. J.*, 1906, **1**, 175.

Concentration of Solution.		Proportion of Dye in Solution.	Proportion of Dye in Paper.
Initial.	Final.	Per cent.	Per cent.
0·014	0·0056	40	60
0·012	0·0024	20	80
0·010	0·0009	9·3	90·7
0·008	0·0003	4	96
0·006	0·00008	1·3	98·7
0·004	—	trace	practically all
0·002	—	trace	practically all

If put into the form of a curve, where the ordinates represent the percentage of dye left in solution and the abscissæ the original concentration of the solution, the curve only approaches the axis (*i.e.* zero concentration) asymptotically. In other words, the more dilute the original solution the more firmly the dye is adsorbed. Similar relations are found to exist between proteins and electrolytes. By continuously washing a protein, *e.g.* gelatin, with distilled water, the removal of electrolytes becomes slower and slower, and it is impossible within finite time to get rid in this way of the last traces of ash.

COLLOIDAL IONS. Although the chemical behaviour of colloids is largely determined by surface phenomena, it is also conditioned by the chemical structure of the colloid molecule and by the charge carried by the latter. A good example of these combinations is presented by globulin, the behaviour of which has been studied by Hardy.* Four states can be recognised both in the solid condition and in solution, viz. globulin itself, compounds with acid or with alkali, and compounds with neutral salt. The amount of acid and alkali combining with the globulin is indeterminate, the effect of adding either acid or alkali to the neutral globulin being to cause a gradual conversion of an opaque, milky suspension into a transparent solution. On drying HCl-globulin, the solid is found to contain all the chlorine used to dissolve it. The acid may therefore be regarded as being in true combination. Both acid and alkali globulins act as electrolytes, the globulin being electrically charged and taking part in the transport of electricity. It is, in fact, present as an ion, charged positively or negatively, according to the reaction. In order to produce the same degree of solution, the concentration of the alkali added must be double that of the acid. The relation of globulin to acids and alkalies is similar to that of the so-called *ampholytes* or amphoteric substances, such as the amino-acids. An amino-acid such as glycine can react as a basic anhydride with other acids thus :

$$CH_2\left\langle\begin{matrix}NH_2\\COOH\end{matrix}\right. + HCl = CH_2\left\langle\begin{matrix}NH_2HCl\\COOH\end{matrix}\right.$$

or as an acid with bases :

$$\begin{matrix}CH_2.NH_2\\|\\COOH\end{matrix} + NaHO = \begin{matrix}CH_2.NH_2\\|\\COONa\end{matrix} + H_2O$$

Like these too, globulin forms soluble compounds with neutral salts. An amphoteric electrolyte thus acts as a base in the presence of a strong acid and as an acid in the presence of a strong base.

According to recent developments of physical chemistry, amino-acids more probably have what is called a *zwitterion* structure: $^+NH_3.R.COO^-$. If this is so, then the

* HARDY. *J. Physiol.*, 1905, 33, 251.

action of acids and alkalies differs from that previously accepted, for the zwitterion is positive at one end and negative at the other. Addition of a strong acid therefore acts, not by enhancing the basic property of the NH_2 group, so causing the molecule to acquire a positive charge, but by depressing the ionisation of the carboxyl, *i.e.*, the negatively charged group:

$$^{+}NH_3.R,COO^{-} + H^{+}\,Cl^{-} = {}^{+}NH_3.R.COOH + Cl^{-}$$

The zwitterion theory can also be applied to polypeptides and proteins.*

From true electrolytes, colloidal solutions differ in the fact that their particles are of varying size according to the conditions in which they exist, and carry varying charges of electricity, whereas an ion such as $Na^{\cdot}$ or Cl' has a mass which is constant for the ion in question and always carries the same electric charge. The charged particles of an acid- or alkali-globulin may be distinguished therefore as *colloidal* or *pseudo-ions*. Whether a protein has a positive or a negative charge depends chiefly on the hydrogen ion concentration of the medium. At a certain H-ion concentration, known as the *iso-electric point*, it carries no charge (or, according to the zwitterion theory, there is complete ionisation of both groups), and it then exhibits maximum precipitability (it often flocculates out), minimum viscosity, minimum osmotic pressure, and minimum imbibition.

The iso-electric *p*H of some important proteins are given below:—

Edestin	6·9
Reduced hæmoglobin	6·8
Oxyhæmoglobin	6·6
Serum globulin	5·4
Egg albumin	4·8
Serum albumin, Casein, Gelatin	4·7

Precipitation of colloids at their iso-electric points is an important method of purification of colloidal substances, or, alternatively, of removing colloidal impurities from mixtures.

Combinations Between Colloids. Besides the compounds between colloids and electrolytes, combination, or at least interaction, takes place between different colloids. Many colloids are precipitated by other colloidal solutions. This effect is always found to occur when the colloidal solutions carry opposite charges. Thus ferric hydrate in colloidal solution is precipitated by colloidal silica or colloidal gold, both colloids being thrown out of solution. On the other hand certain colloids may exercise a protective influence on other colloidal solutions. Thus, as Faraday first showed, colloidal gold is much more stable in the presence of a little gelatin. The colloids of serum can dissolve a considerable amount of purified globulin. Although the latter alone shows a drift in the electric field, the resulting solution is quite unaffected by the passage of a current through it. In these cases the protective colloids carry no charge, but the same protective effect may be observed if a large excess of *e.g.* an electro-positive colloid be added to an electro-negative colloid. This interaction between different colloids probably plays an important part in many physiological phenomena. We have reason to believe that the reactions between toxin and antitoxin and between enzyme and substrate, which we shall study later, are of this

* Birch and Harris. *Bioch. Journ.* 1930, **24**, 1080; Richardson. *Proc. Roy. Soc.*, 1934, **115B**, 121.

character, and that the compounds formed belong to the class of adsorption combinations.

In these combinations, although the chemical nature of the colloidal molecules is concerned, there is an absence of definite equilibrium points such as we are accustomed to in most chemical reactions. The inertia of the system and the large size of the molecules determine the occurrence of false equilibria and of delayed reaction, so that the condition and behaviour of a colloidal system at any moment are determined, not only by the quantitative relations of its components but also by the past history of the system.

THE COAGULATION OF COLLOIDS

Most colloidal solutions are unstable, and the relations between the suspended particle or molecule and the surrounding fluid may be upset by slight changes of reaction or the presence of minute traces of salts. As a result the hydrosol is destroyed, the suspended particles aggregating to form a precipitate or a gel, the result varying according to the nature of the colloid and its concentration. Thus gelatin changes from the condition of hydrosol to hydrogel with fall of temperature. On the other hand, by adding calcium chloride to an alkaline solution of casein, we obtain a mixture which sets to a jelly on warming but becomes fluid again on cooling. Other agencies may lead to the production of changes which are irreversible.

If a solution of egg-albumin or globulin be heated in neutral or slightly acid medium and in the presence of neutral salt, the whole of it is thrown down in an insoluble form. This coagulated protein is insoluble in dilute acids or alkalies and is said to be *denatured.* With concentrated solutions of protein, heat coagulation results in the formation of a gel. In dilute solutions the result is the production of a flocculent precipitate.

Another method is the so-called mechanical coagulation. If a solution of globulin or albumin be violently shaken, a shreddy precipitate makes its appearance in the solution, and by prolonged shaking it is possible to throw down 80 or 90 per cent. of the dissolved protein in the coagulated form. Ramsden has shown that this mechanical coagulation is a surface phenomenon. In the case of proteins the molecules at the surface are so closely packed together that they form an actual solid pellicle. When the solution is violently shaken, new surfaces are constantly being formed, and as the older surfaces are withdrawn into the fluid, the solid pellicle on them is rolled up into a fine shred of coagulated protein.

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CHAPTER VII

THE PASSAGE OF WATER AND SOLUTES THROUGH MEMBRANES

THE heterogeneous structure of the body involves the presence of surfaces of separation, which may be of any order of size, molecular, microscopic, or evident on simple dissection. In the single cell the membrane bounding the cell and separating it from its environment is as a rule invisible under the microscope. Similar membranes must be assumed to be bounding the cell nucleus. In other cases the membrane is of a definite thickness: it may be apparently structureless and homogeneous such as the elastic lamina in the back part of the cornea of the eye, or it may be made up of cells of various shapes. The bounding membrane of the blood capillaries or of the lung alveoli is composed of very thin flat cells; or the membrane may consist of cubical or columnar cells, as in those forming the lining membrane of the small intestine or the essential part of secreting glands. In such a cellular membrane any transference across the membrane may be regulated by the cells themselves, taking up a substance on one side and turning it out on the other, in many cases after having undergone modification. It is impossible, however, to investigate the part played by these cells in the transference of substances across a membrane unless we know what factors determine the passage of water and dissolved substances across membranes which do not take an active part in the process of transference. Such a membrane we can imagine of any thickness, from one molecule upwards since the thickness will affect only the rate of passage of substances across it. Surface films, one molecule in thickness, are readily formed on liquids, *e.g.* when fatty acids are placed on water. In such a case the molecules are arranged vertically, with the carboxyl groups next the water.* Similar *mono-molecular films* are also in all probability formed at the surface of colloid solutions and, as a general case, at surfaces where adsorption occurs.

The following factors may be concerned in causing the movement of water or dissolved substances across a membrane :—

(1) HYDROSTATIC PRESSURE. The significance of this is familiar in the ordinary process of filtration. A filter pump merely increases the difference of pressure between the two sides of the filtering membrane. The denser the membrane the greater will be the difference of pressure required to effect a passage at a certain date.

(2) DIFFUSION AND OSMOSIS. In solution the molecules can move freely only within the limits of the solution. Diffusion is due to this movement. In gases the rate of diffusion varies inversely as the square root of the density of gas. We also find differences between the rates of diffusion

* ADAM. "The Physics and Chemistry of Surfaces," 1930 (Clarendon Press, Oxford); LEATHES, *Lancet*, 1925, **103**, (1), 853.

of dissolved substances—determined in all probability by the weight and size of the individual molecules, although the relation between molecular weight and rate of diffusion is not so simple as in gases.

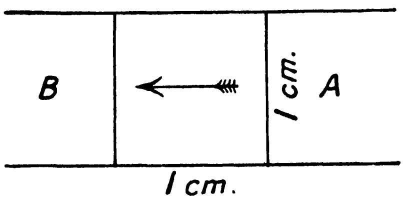

Fig. 24.

The diffusibility of a substance is given by its *diffusion coefficient.* It we take a cylindrical mass of solution which is one centimetre long and has a sectional area of one square centimetre (Fig. 24), and maintain a constant difference of concentration between A and B = 1, the diffusion coefficient is the amount of substance which diffuses in a unit of time from A to B. Thus the statement that the diffusion coefficient of urea is 0 810 at 7·5° C. denotes that, if A be continually filled with a 1 per cent. solution of urea, while in B a constant current of distilled water is kept up so as to maintain the concentration at zero, in the course of a day 0 810 gramme of urea will pass from A to B through the cylinder of one centimetre in length and one square centimetre in cross section. The determination of these diffusion coefficients presents many difficulties. The task is, however, rendered easier by the fact, first ascertained by Graham, that diffusion of salts occurs as rapidly through a solid jelly of gelatine or agar-agar as through water. It is therefore possible to make the plug in the diagram solid by the admixture of one of these two substances, and to maintain a constant concentration on the two sides of it by the circulation of fluid without affecting the rate of diffusion through the cylinder by setting up accidental currents.

If, of two fluids in contact, the one fluid contains, say, sugar and the other fluid salt, these will both move in opposite directions until homogeneity is attained. There may, however, be different initial distributions and temporary alterations of the distribution of the total fluid on the two sides of the membrane, due to varying rates of diffusion of the dissolved substances.

If in Fig. 25 for instance A contains a solution of sugar and B an isosmotic solution of NaCl, separated by a membrane freely permeable both to water and to these substances, the salt will diffuse from B to A more rapidly than the sugar will diffuse from A into B. There will thus be a tendency to a rise of the total osmotic pressure in A and to a passage of water from B into A. This will cause an initial increase of the total fluid in A and a diminution in B or, if both compartments are closed (Fig. 26), the pressure

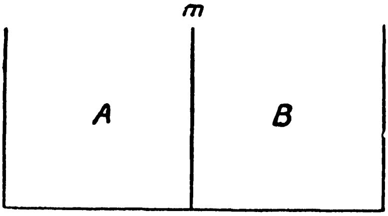

Fig. 25.

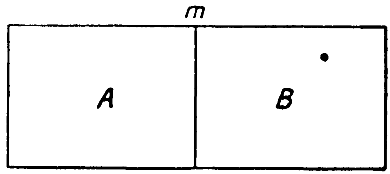

Fig. 26.

in A will rise above the pressure in B. This rise of pressure in A will cause a *filtration of the whole fluid* from A to B, so that in time we shall attain equilibrium both of pressure and of contents between A and B. The time taken to attain this equilibrium will vary with the thickness of the membrane, and with a membrane of molecular dimensions and compartments of microscopic size the equilibrium will be attained almost instantly, so that the effects of 'initial osmosis' may be disregarded.

The physico-chemical conditions for passage of water and salts across membranes have been studied in detail by Schreinemakers * and found to be complex, since when two solutes are present together in the solution on either side of a given membrane, the resultant transfer of water or of either solute depends on many factors. If the membrane is truly semi-permeable in the classical sense, *i.e.* is only permeable for water, equilibrium between two-solute mixtures will be reached when the two solutions have the same osmotic pressures, but their composition will probably not be the same. If the membrane is permeable to both solutes as well as to water, then when equilibrium is reached the composition on both sides will be identical, though the changes undergone in reaching such equality (or the " osmotic path ") will be complicated and not predictable on general theoretical grounds, though modified by the properties of the membrane and the original composition and amounts of the solutions. A substance may at one phase of the diffusion pass in one direction and afterwards in the opposite one. In general terms, when n substances can pass a membrane, then there will be 2^n different types of diffusion, of which one is impossible, and $2^n - 1$ possible. For instance, if there are six substances, there will be $2^6 - 1 = 63$ actually possible types of diffusion (*i.e.* when each substance may move either to right or to left that is counted as two types). Which of the possible types will actually occur depends on the properties of the membrane, and these cannot be predicted.

(3) Electrical Difference of Potential. This factor probably plays an important part in the transference of dissolved substances across membranes in the body. Just as differences of hydrostatic pressure determine movements of a fluid *en masse*, differences of electric pressure will serve as a driving force for the charged constituents of fluids. These may be actual particles, *e.g.* oil droplets or bacteria, the ultra-microscopic particles of colloids or the ions derived from the electrolytic dissociation of dissolved molecules. The passage depends on the fact that any charged particle will move in an electric field, the positive particle moving towards the negative pole while the negatively charged particle moves towards the positive pole. The electricity passing through the fluid is in fact carried by the particles which are thus set into movement. If a current is passed across a membrane immersed in an electrolyte, as in the diagram (Fig. 25), the final result will be a chemical difference on the two sides and therefore a permanent difference of potential which will tend, on breaking the current, to drive the ions in the opposite direction and to re-establish equilibrium on the two sides. The process of passage of a current across a membrane is said thus to give rise to *polarisation*, and after the passage of the current the membrane is polarised, *i.e.* presents a difference of potential between its two sides.

The presence of a membrane impermeable to the colloidal constituent of an ionised colloidal salt will affect the passage of a current through the membrane in one direction, so that the phenomenon of ' irreciprocal conductivity ' is presented. Thus if in Fig. 25 A contains the potassium salt of hæmoglobin and B contains potassium chloride, a current can pass from A to B, since it is carried from A to B by the potassium ions which can pass freely through the membrane, and from B to A by the electro-negative ions, namely, chlorine, An application of this method called *electro-dialysis*, is in fact one of the best ways of freeing protein solutions from adsorbed basic ions, If, however, B be made the positive pole, no current can pass because the electro-negative ion, namely hæmoglobin, cannot pass through the membrane *m*. If we take an arrangement with three compartments A, B and C, of which only B contains a protein salt, the passage of a current will be hindered in both directions, since, whichever the direction of the current, the protein molecule will be required to carry the negative electricity either into A or into C. This resistance to the passage of a current will be still more marked if the membrane is impermeable also to certain inorganic ions. It is on this account that whole blood presents a greater resistance to the passage of a current than does the serum, each of the red blood corpuscles serving as a non-conductor and therefore diminishing the total area through which the current can pass.

In nearly every case the hindrance to migration of one or more ions by the membrane will prevent the passage of other associated ions to which by themselves the membrane would be freely permeable. These ions will travel an infinitesimal distance through the membrane till they are held back by the electrostatic attraction of the oppositely charged ions which cannot pass. This electrostatic tension will give rise to a charge on the surface of the cell, and in nearly every case free swimming cells such as red blood corpuscles are found to be charged differently from the solution in which they are

* Schreinemakers. *J. Gen. Physiol.*, 1928, **11**, 701 ; 1929, **12**, 555 ; 1930, **13**, 335.

suspended. In most cases this charge is negative, so that, if a current be passed through blood, the red blood corpuscles will tend to travel towards the positive pole.

A charge on a membrane may also cause water to pass with an electric current through the membrane. This may be explained as follows: A charged particle, if free to move, will travel to the pole with the opposite charge; thus clay is negatively charged and migrates to the anode. Suppose now in Fig. 25 that *m* is made of clay and that electrodes are immersed in the fluid, the anode in A and the cathode in B. Now since the clay partition is fixed the water moves instead, viz. to the negative pole, so that the volume in B is increased. This phenomenon is called *electro-endosmosis.* With a gelatine membrane, the charge depends on the reaction of the solution. If this is alkaline, the gelatin is, like clay, negatively charged, but if acid it is positively charged and the direction of the flow is reversed.

(4) Permeability of the Membrane. The nature of the membrane cannot add to the force concerned in the transference of water or dissolved substances across it: it can only serve as a limiting condition for the action

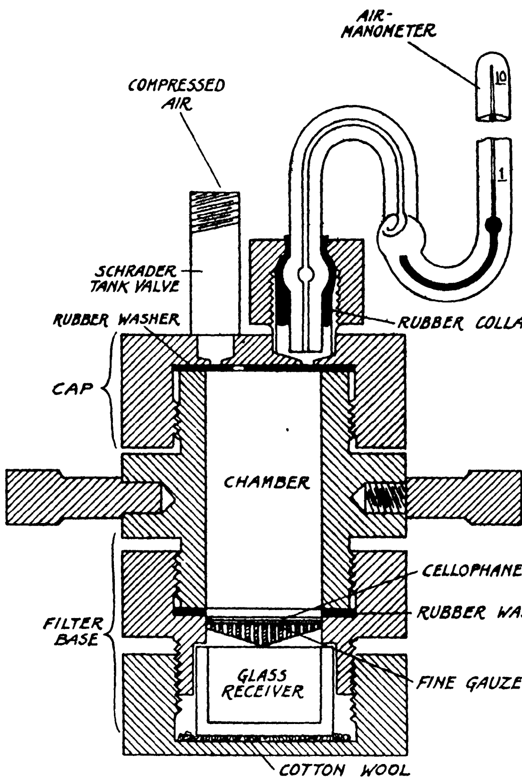

Fig. 27. Apparatus for ultra-filtration. The filtering membrane is cellophane. The upper chamber contains the colloid solution, from which a colloid-free filtrate is forced through into the lower chamber by air pressure. (Aitken and Kay, *J. Physiol.*, 1927, **62**, Proc. xxix.)

of the forces just enumerated. How these forces will act in regard to different kinds of membrane will depend on the permeability of the membrane, and this again on its physical and chemical structure. The membrane may be absolutely impermeable, as in the case of those surrounding certain eggs, and in plants as a means of protection for seeds. In most cases, however, the permeability is relative. Filter paper, for instance, permits the passage both

of water and of all dissolved substances. Different kinds of filter paper allow passage with varying degrees of rapidity, depending on the fineness of the holes through which the fluid can pass. The same difference may be observed in porous structures, such as the Berkefeld filter, which allow all dissolved substances to pass but keep back most bacteria. On the other hand the very smallest micro-organisms, the so-called filter passers, will traverse the pores of a Berkefeld filter. Most gels, such as gelatin and collodion are of porous structure, and collodion membranes may be obtained of varying degrees of permeability according to their mode of preparation * and the concentration of the collodion in the membrane. Such membranes are used for the process kuown as *ultra-filtration.* The membranes of loosest texture will allow proteins such as albumin to pass, in addition to crystalloids. On the other hand the denser collodion membranes, or cellophane, will keep back all colloid constituents and allow only water and crystalloids to pass. If, for instance, serum be placed on one side of such a membrane, under pressure a filtrate will be obtained containing the water, salts, urea and other crystalloid constituents of he serum (Fig. 27). They are also made use of when it is desired to free a colloidal solution from salts, in the process known as *dialysis.* If a fluid containing both colloids and crystalloids in solution, *e.g.* blood serum, be enclosed in a tube of vegetable parchment or collodion which is hung up in a large bulk of distilled water (Fig. 28), all the salts diffuse out, and if the water be frequently changed, we obtain finally a fluid within the 'dialyser' free from salts and other crystalloid substances, but containing the whole of the colloids originally present.

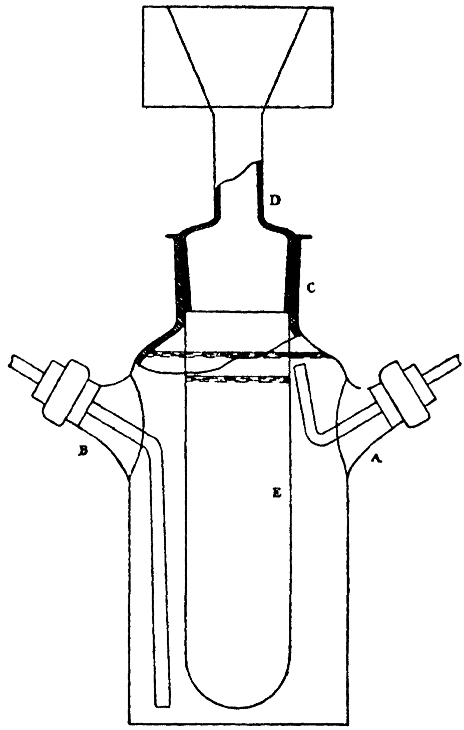

FIG. 28. Dialysing Apparatus. The collodion tube E is fastened to the glass stopper D which fits airtight into the neck of the bottle. Sterile water is introduced into the bottle from a reservoir through B, and the colloid for dialysis poured into E A partial vacuum is then made in the bottle by exhaustion at A; this opposes the osmotic pressure of the solution in E and so prevents its dilution, or if great enough may even concentrate it by ultra-filtration. (JESSNE HANSEN.)

If a certain pressure is put on the contents of the dialyser, or (as in Fig. 28) suction on the outside, fluid also passes out by ultra-filtration, but there is a certain limiting pressure below which no filtration will take place. This limiting pressure is equivalent to the osmotic pressure of the colloids in solution, and varies according to the state of aggregation of the colloid, *i.e.* to the number of molecules, simple or compound, of the colloid which is present. In blood serum it is equal to about 30 mm. Hg. If the difference of pressure on the two sides of the membrane is less than this amount, water

* PIERCE. *J. Biol. Chem.*, 1927, **75**, 795; DESAI, *Bioch. J* 1930, **24**, 1897.

and salts will pass from the outside to the inside of the membrane and the serum will be diluted.

A still greater degree of impermeability is obtained with "semi-permeable" membranes.

No completely semi-permeable membrane is known, *i.e.* one which allows the passage of water but is impermeable to all dissolved substances. The best known artificial semi-permeable membrane is that composed of copper ferrocyanide, supported in the meshes of a clay cell. This membrane is impermeable to copper salts, to ferrocyanides, to sugar and to certain other dissolved substances: it permits KCl, however, to pass freely. Its semi-permeability may be increased by depositing AgCl, when it becomes impermeable also to potassium chloride. Such membranes are of much interest since, as already mentioned, the surfaces of all cells show in greater or less degree the phenomenon of semi-permeability. Thus, red blood corpuscles are impermeable to most kations, such as Na, K and Ca, but allow the passage of urea, CO_2, chlorine, OH ions as well as NH_4 and glucose (slowly).*

Finally, we may have a form of permeability which depends on the solubility of the solute in the membrane itself, or on the formation of reversible compounds between the solute and the membrane. Thus india-rubber dissolves carbon dioxide. If a rubber balloon containing air be placed in a vessel filled with carbon dioxide, the balloon rapidly expands and may even burst. The carbon dioxide molecules are dissolved in the rubber and diffuse from the other side into the air inside the bag. The molecules of nitrogen and oxygen cannot pass in the reverse direction, so that the balloon expands. It has been supposed that phenomena of a similar nature may play a part in determining the peculiarly specific permeability of cell membranes. Cell surfaces are freely permeable to a number of organic substances, such as the alcohols which are soluble in lipides. It has therefore been suggested by Overton that the limiting membrane is lipoidal in nature, formed, *e.g.* of a mixture of lecithin and cholesterol. It is true that these substances take part in the formation of the membrane, which may be regarded as a surface aggregation of molecules forming part of the contents of the protoplasm within the cell; but it seems certain that proteins also take part in the formation of this membrane, and that in some way its integrity is dependent on the presence of certain salts or ions in the outer fluid which may be adsorbed on the external surface of the membrane and so contribute to its qualities. Thus many cells, such as egg cells, muscle cells, entirely alter their permeability if immersed for a time in a fluid free from Ca salts, and under these circumstances may become permeable to substances such as KCl, which they do not ordinarily allow to pass. Other objections to Overton's theory have been mentioned on p. 20.

The permeability of living membranes often shows peculiarities, which depend on the structure and previous history of the membrane. For instance, Reid † found that fresh frog's skin allows water to pass only from without inwards, and Wertheimer,‡ found that in frog's skin sodium salts and acid dyes only pass from without inwards, while glucose and basic dyes only pass outwards.

The explanation of irreciprocal permeability probably varies from instance to instance, but is mostly unexplained. As regards water, it is thought to be related to the so-called anomalous osmosis which depends on the membrane being electrically charged.

* Hsu. *J. Physiol.*, 1935, **84**, **29 P**.
† Reid. *J. Physiol.*, 1890, **11**, 312.
‡ Wertheimer. *Pfluger's Arch.*, 1923, **199**, 383.

As a result of this phenomenon it was shown by Loeb that water could pass in a direction contrary to the osmotic gradient.

The surface membrane of a cell differs, moreover, from artificial semi-permeable membranes in that its permeability varies according to the state of the cell itself. Thus, activity of a cell is always attended with an increased permeability of its surface membrane, and this change may be an important factor in the execution of its functions. It is this dependence of permeability on cell function that must be made responsible for the wide differences observed between the composition of a cell and that of its surrounding medium. The red blood corpuscles, for instance, are rich in potassium salts and may contain no sodium salts, whereas the reverse holds good for the blood plasma in which they are bathed.

Some suggestions as to how these changes in permeability are effected have been made on p. 20.

Not only does the function of the cell determine the properties of its surface, but the reverse holds true. Cell activities seem to be determined to a very large extent by alterations in the amounts of substances in the surrounding medium even though they cannot penetrate the limiting membrane of the cell. This is especially well marked in the case of contractile tissues such as the heart where the normal beat depends on the presence in the surrounding fluid of K, Na and Ca ions. None of these can penetrate the cell membrane: they can merely be adsorbed on the cell surface, and it is their presence there which in some way or other modifies all the functional activities of the muscle cell.

(5) MEMBRANE EQUILIBRIA. If a colloid is in the form of a compound with either acid or base, which undergoes ionic dissociation, certain interesting results are possible.* For instance, Congo red is a sodium salt of a colloid acid. If this be placed in an osmometer which is immersed in water, a certain osmotic pressure is developed. Although sodium ions can pass readily through the membrane of the osmometer, they carry a positive charge, so that if they broke away from their combination with Congo red acid, the fluid in the osmometer would become negatively charged. The sodium ions are thus held in the immediate neighbourhood of the membrane by the electrostatic attraction between Congo red acid ions and the sodium ions. If we add sodium chloride to either the inner or outer fluid, the osmotic pressure is found to fall if time be allowed for equilibrium to be established between the two sides of the membrane. At this point the outer fluid, which is free from dye, contains a larger percentage of chloride than the inner fluid. This difference is permanent and is more marked the greater the concentration of the dye. In the following Table are given the concentrations of the two fluids with different percentages of salt, the numbers indicating the litres to which each gramme molecule of the salt is diluted.

Dye.	Chlorine.	
	Inside.	*Outside.*
30	52	30
30	465	73·6
30	5500	180
100	32·9	29·5

Donnan † independently showed that these results must occur from thermodynamic considerations, so that their general statement is spoken of as the ‘Donnan equilibrium.’

When a saline solution contained in a collodion sac is suspended in distilled water, the simple laws of diffusion suffice to explain the final equal concentrations of salt reached on both sides of this permeable membrane. If, however, a sodium salt of a non-diffusible anion (R) be placed on one side of a dialysing membrane and water on the other side, simple osmosis does

* BAYLISS, W. M. *Proc. Roy. Soc.*, 1909, **81B**, 269; 1911, **84B**, 229.
† DONNAN. *Ztschr. f. Elektrochem.*, 1911, **17**, 572.

not suffice to explain the final state of equilibrium. The Na will tend to pass through the membrane, but this can only take place if an equivalent quantity of OH (from the water) diffuses in the same direction. Thus :

Initial State		‖	*Final State*	
$Na^{\cdot}$	H_2O	‖	$Na^{\cdot}$	$Na^{\cdot}$
R'		‖	$H^{\cdot}$	OH'
		‖	R'	

The water will become alkaline, while the solution in the left compartment will become less alkaline. If, on the other hand, a salt, say a chloride, of a non-diffusible kation, were placed on the left-hand side, then the water outside would become acid, and the solution on the left less acid.

Again if NaR be placed on one side of the membrane and NaCl on the other side, equilibrium will be attained only when the products of the concentrations of [Na] and [Cl] are the same on opposite sides of the membrane.

Initial State		‖	*Final State*	
$Na^{\cdot}$	$Na^{\cdot}$	‖	$Na^{\cdot}$	$Na^{\cdot}$
		‖	Cl'	Cl'
R'	Cl'	‖	R'	

$$[Na]_1 \times [Cl]_1 = [Na]_2 \times [Cl]_2$$

This can only occur if $[Na^{\cdot}]_1 > [Na^{\cdot}]_2$ and $[Cl']_1 < [Cl']_2$.

This difference in the concentration of the diffusible ions on opposite sides of the membrane gives rise to a potential difference which is equal to $58 \log. \frac{[Cl]^1}{[Cl]_2}$ millivolts.

Since many of the compounds in the body are of the form NaR, *e.g.* sodium proteinate, and the living tissues abound in membranes impermeable to colloids, Donnan's theory of ' membrane equilibria ' must have a wide application in physiology. For example, it provides an explanation of the distribution of ions, and also suggests how strongly acid secretions, such as the gastric juice might be formed.

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CHAPTER VIII

ELECTRICAL CHANGES IN LIVING TISSUES

THE material composing living cells is permeated throughout with water containing electrolytes in solution. All salts undergo ionic dissociation in watery solution. When an electric current is passed through a tissue it is carried by the charged ions formed by the dissociation of the salts. In addition to these charged inorganic ions, the cell protoplasm contains various colloidal particles which in many cases are themselves charged. By the presence of these colloidal ions, marked differences may be caused in the distribution of the inorganic ions owing to the establishment of Donnan equilibria. Any unequal distribution of the charged ions in a tissue, or on the two sides of a membrane, will give rise to corresponding differences of electric potential between the parts concerned, and any further change in this distribution will further modify these potentials. It is therefore not surprising that practically every functional change in a tissue has been shown to be associated with the production of differences of electrical potential. Thus, all parts of an uninjured muscle are isopotential, and any two points may be led off to a galvanometer without any current being observed. If, however, one part of the muscle be excited, or injured, it will be found that, on leading off from this point and a point on the resting uninjured surface to a galvanometer, a current flows through the latter from the uninjured to the injured surface. Every beat of the heart, every twitch of a muscle, every state of secretion of a gland is associated in the same way with electrical changes. The electrical changes accompanying activity have the same general character, the excited part being found to be galvanometrically negative with reference to any other part of the tissue which is at rest.

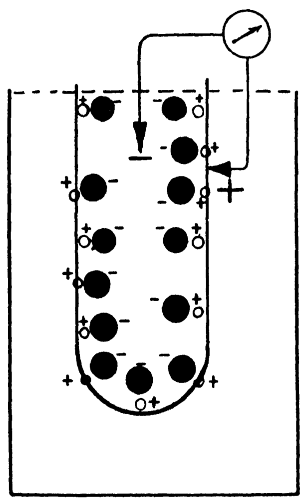

FIG. 29.

Various explanations have from time to time been advanced to explain these phenomena. The one most in favour at present is the membrane potential theory.*

We have seen that when two solutions are separated by a membrane impermeable to one or more of its ions and permeable to others, the membrane becomes charged. Thus, suppose we have, as in Fig. 29, a collodion

* OSTWALD, *Z. physik. Chem.*, 1890, **6**, 71; LILLIE, "Protoplasmic Action and Nervous Action," 1923 (Univ. Chic. Press); LABES, *Arch. f. exp. Path.*, 1927, **125**, 29; *Z. f. Biol.*, 1932, **93**, 42; LABES *et al.*, *Ar. f. exp. Path.*, 1927, **126** 284, 352; *Pflüger's Arch.*, 1932, **230**, 738.

sac filled with an alkaline solution of a protein, *i.e.* with the protein in the form of a salt with a base, and immersed in water or saline solution.

The proteinate solution will be ionised into the basic ion B^+ and the large protein acid ion Pr^-, and the latter cannot pass through the collodion; therefore, although the collodion is permeable to the basic ion, this cannot pass through it either, because it is kept back by the attraction of the oppositely charged protein ion. But, owing to the tendency of the B^+ ion to penetrate the membrane, the two ions are arranged in an orderly fashion, the negatively charged ones innermost and the positively charged ones next to the membrane or perhaps just within its substance. The membrane is thus *polarised* or charged, the outer surface positively, so that if we lead off from its outer and inner surfaces a potential difference can be demonstrated and shown to agree with theoretical calculations.

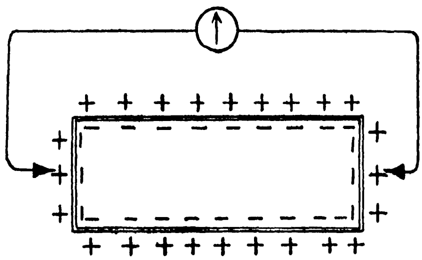

FIG. 30.

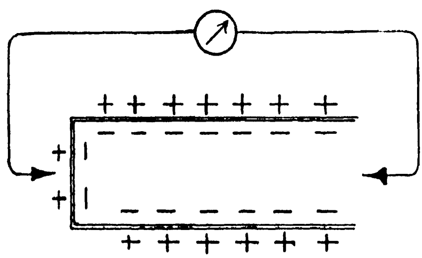

FIG. 31

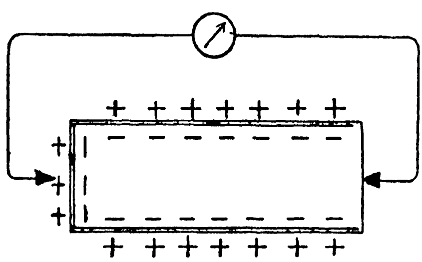

FIG. 32.

FIGS. 30, 31, 32. To show the origin of bioelectric potentials. The double line round the cell indicates a polarised membrane, the single line in Fig. a depolarised one.

A similar state of affairs is present in living cells, which are likewise filled with protein and other colloidal substances in combination, partially ionised, and separated from the surrounding fluid medium by a membrane impermeable to colloids. We therefore get the condition shown in Fig. 30 in which the outside of the cell, or cells, is positively charged, so that if we connect any two points on its surface no current flows. Now suppose the cell is injured, we get, at the injured point, direct access to the negatively charged contents of the cells, as in Fig. 31, and so a current will flow through a galvanometer connected with injured and uninjured surfaces. Similarly, if the cell, or part of it, becomes active, changes occur in the membrane by which it becomes *depolarised* at the site of activity and so in that region is uncharged; again, it will be relatively negative to (*i.e.* less positive than) the resting uninjured surface, as shown in Fig. 32.

Although we speak of currents flowing through galvanometers in discussing such subjects as the above, it will be clear that as soon as any appreciable current does flow, the equilibrium will be upset, and the current flow will soon cease. In practice, the galvanometer circuit must be of very high resistance, or better, be replaced by some form of electrometer circuit by which potential differences are measured without drawing off any current at all.

CHAPTER IX

CATALYTIC AND ENZYME ACTION

ALL the events of life are accompanied and conditioned by chemical changes of the most varied character. Syntheses by ordinary laboratory methods fall far short of the process as it actually occurs in the living cell, both in completeness of the reaction and in the ease with which it can be effected. Many of the chemical transformations effected by living cells we have so far been quite unable to imitate. The distinguishing characteristics of the chemical changes wrought by the living cell are :

(1) The rapidity with which they are effected at ordinary temperatures.

(2) The specific direction of the process, which is therefore almost complete, with a surprising absence of side reactions. This characteristic may, however, be regarded as a consequence of the first, since an increase in the velocity of any given reaction will determine a preponderance of this reaction over all others.

(3) The production of optically active substances when possible.

All chemical changes in living beings take place in a watery medium. When the cell is deprived of water, it is either killed or enters into a state of inactivity. But water plays a direct part in many of the reactions which take place.

Apparently the molecule may act as a whole, or the H and OH ions may be separately involved. When the molecule acts as a whole, *e.g.* in hydrolysis, the water is added to the molecule on which it acts, with the production of a hydrated product or with a splitting of the original molecule into two parts as a result. The rapidity with which these changes occur in the body is very striking. Thus if we take a solution of boiled starch into the mouth, keep it there for twenty seconds and then eject it, the fluid is found to contain no starch but to contain maltose. In this case there has been a taking up of water molecules by the starch, so that the interaction of water appears in the equation—

$$2\ (C_6H_{10}O_5)_n + nH_2O = nC_{12}H_{22}O_{11}$$

On the other hand, a solution of boiled starch in water may be left for many weeks or months without undergoing this change, which, however, can be induced by heating the fluid in an autoclave to 120° C. for several hours. There must be some means by which the action of the water on the starch is quickened to such an extent that the process of hydrolysis occurs at the temperature of the body even more rapidly than it occurs in the autoclave. In the body this process of quickening the velocity of chemical change in certain definite directions is carried out by means of *enzymes*.

ENZYMES

Under the name enzymes we include a number of complex colloidal organic catalysts formed by living cells, and capable of exerting their action after removal from the cells. Minimal quantities of enzyme can induce

chemical changes involving almost unlimited quantities of other substances, the only result of increasing the quantity of enzyme being to quicken the rate of the change. In this respect their action is similar to that of a whole class of bodies which are spoken of as *catalysers* or *catalysts*. A catalyser is a substance which will increase (or diminish) the velocity of a reaction without adding in any way to the energy changes involved in the reaction, or taking any part in the formation of the end products.

Enzymes may often be extracted from the tissues with water, glycerol, salt solutions, or dilute alcohol. Such soluble enzymes are called *lyo-enzymes*. Others are insoluble, either because they are bound chemically to the cell cytoplasm (*desmo-enzymes*), or because they are adsorbed to a constituent (*endo-enzymes*).* The difficulty in their isolation is increased by the fact that all of them are colloidal, and present the tendency common to all colloids of being adsorbed to other colloidal matter as well as to precipitates. A common method of obtaining a concentrated preparation of an enzyme is to add to its solution a strongly adsorbing colloidal precipitate, such as alumina or kaolin. Kaolin, being negatively charged, adsorbs those enzymes which have basic characters, while alumima, a positively charged colloid, adsorbs acid substances. The enzyme is carried down on the precipitate and may be obtained in solution by " elution " with water or dilute acid or alkali. By fractional adsorption with different adsorbents, enzymes may be greatly concentrated and also separated from one another. In this way many enzymes formerly considered as entities have been shown to consist of mixtures of two or more enzymes, and some have been obtained in a pure crystalline state. A further difficulty in their preparation lies in the unstable character of many members of the group, so that every act of precipitation of an enzyme tends to rob it of some of its powers.

The list on p. 95 represents some of the enzymes whose existence has been definitely established in the animal body.

The substance which is chemically altered as a result of enzyme action is called the *substrate*. Enzymes may be grouped into two great classes, according to their actions. The first class, the *Hydrolases*, break up larger molecules into smaller ones by effecting simple hydrolysis. The second class, the *Desmolases* break up the molecules more profoundly, as by oxidation.

The Hydrolases include the groups of Esterases, Carbohydrases, Amidases, and Proteases. The Desmolases are less clearly divided into the groups Dehydrases, Carboxylases, Oxidases, Peroxidases and Catalases.

Many new enzymes are emerging with increase in our knowledge of cellular metabolism. The list which is here given suffices to show how great a part these bodies must play in the normal processes of life.

It is important to note that all the changes wrought by enzymes can be effected by ordinary chemical means. Thus the disaccharides can be made to take up a molecule of water and undergo conversion into monosaccharides. A solution of maltose at ordinary temperatures undergoes practically no change. If the solution be warmed, a slow process of hydration takes place which is quickened by rise of temperature, so that if the solution be heated under pressure to, say, 110° C., hydrolysis occurs with considerable rapidity. If, however, a little maltase be added to the solution, the change of maltose into glucose takes place rapidly at a temperature of 30° C. The action of the enzyme is to quicken a process of hydrolysis which without its presence would take an infinity of time for its accomplishment.

* BAMANN AND SALZES. *Ergeln. d. Enzym. forsch,* 1938, 1, 28.

TABLE OF IMPORTANT ENZYMES.

Group.	Member.	Substrate.	Products.
I. HYDROLASES—			
ESTERASES	Lipase	Fats	Fatty acids + glycerol
	Esterase	Simple esters	Hydrolysis products
	Phosphatase	Phosphoric esters	,, ,,
CARBOHYDRASES	Sucrase	Sucrose	Glucose + fructose
	Maltase	Maltose	Glucose
	Lactase	Lactose	Glucose + galactose
	Amylase	Starch	Dextrin and maltose
	Glycogenase	Glycogen	Glucose
	Nucleases	Nucleic acids	Splits off carbohydrate
	Glyoxalase	Pyruvic aldehyde	Lactic acid
AMIDASES or amino-acyclases	Urease	Urea	Ammonium carbonate
	Histozyme	Hippuric acid	Benzoic acid + glycine
	Arginase	Arginine	Urea + ornithine
	Purinamidases	Purin bodies	Deamination products
PROTEASES	Peptidases (*e.g.* erepsin).	Peptides	Amino-acids
	Proteinases		
	(*a*) Pepsinases (pepsin)	Proteins (as cations)	Proteoses and peptones
	(*b*) Tryptases (trypsin)	,, (as anions)	Peptones and amino-acids
	(*c*) Autolytic proteinases (cathepsin)	,,	Amino-Acids
	(*d*) Rennin (?)	Caseinogen	Casein
II. DESMOLASES—			
DEHYDROGENASES	Aldehydrase	Aldehyde	Acid + alcohol
	Lactic dehydrase	Lactic acid	Pyruvic acid
	Succinodehydrase	Succinic acid	Fumaric acid
	Purindehydrase	Uric acid	Allantoin
CARBOXYLASES	—	Keto-acids	Aldehyde + CO_2
OXIDASES	—	Various	Various
PEROXIDASES	—	Phenols + H_2O_2	Various
CATALASES	—	Hydrogen peroxide	$H_2O + O_2$
CARBONIC ANHYDRASE		H_2CO_3	$CO_2 + H_2O$

The enzymes are remarkably specific in the substrates on which they can act. Thus amylase acts on starch and not on fat or protein. In most instances the specificity is still narrower. Whereas all the disaccharides are converted by acids into the corresponding monosaccharides, the enzyme invertase acts only on cane sugar and has no action on maltose or lactose, each of which requires a specific enzyme (maltase, lactase) to effect its hydrolysis. But we find many examples of a restricted action even among inorganic catalysers.

The specificity of enzymes varies greatly and shows many peculiarities. Absolute specificity is shown as between enzymes of different groups : *e.g.* lipases will not act on proteins. In other directions, within their groups, the specificity is variable, and is probably related to particular chemical groups in the substrate : thus lipases will also hydrolyse many other esters besides fats. It has been found that, whereas maltase splits up all the α-glucosides, it has no power on the β-glucosides : that is to say, maltase will fit into a molecule of a certain configuration, but is powerless to affect a molecule which differs from the first only in its stereochemical structure. On the other hand emulsin, which breaks up β-glucosides, has no influence on α-glucosides. This specific affinity of the enzymes for optically active groups of bodies suggests that the enzyme itself may be optically active.

ENZYMES AS CATALYSERS. Whereas the influence of most catalysers on the velocity of a reaction increases rapidly with rise of tempera-

ture, in the case of enzymes this increase occurs only up to a certain point (the temperature coefficient varying, up to this point, from 1 to 2). This point is spoken of as the *optimum temperature* of the enzyme action. If the mixture be heated above this point, the action of the enzyme rapidly slows off and then ceases owing to destruction of the enzyme. The same applies to those inorganic catalysers whose physical state is susceptible, like that of the enzymes, to the action of heat. Thus with colloidal platinum the reaction presents an optimum temperature, owing to the fact that the colloidal platinum is altered, coagulated and thrown out of solution when this is heated to near boiling-point.

Since the action of enzymes, like that of catalysts, consists essentially in the quickening up of processes which would otherwise occur at an infinitely slow rate, many investigations have dealt with the velocity of the reaction induced by the action of an enzyme.

It has been found that the velocity of an enzyme action is accelerated by rise of temperature (up to the optimum) by an optimal pH, and by increase in concentration of substrate or of enzyme. Conversely it is inhibited by accumulation of the end-products of the reaction, and hence the action is probably a special case of the laws of mass-action.

Enzymes, which are all colloidal, cannot be dealt with in the same way as the simpler catalysts. In many cases the substrate, *e g.* starch or protein, is also colloidal, and the combination therefore falls into the class of combinations between colloids. In this we have an interaction between two substances in which the adsorption by the surfaces of the molecules of one or both substances plays an important part, though this adsorption is itself determined or modified by the chemical configuration of the molecules.

Various methods may be adopted for the study of the velocity of enzyme action. Thus, for the decomposition of hydrogen peroxide by blood catalase we can measure either the rate at which oxygen is liberated, or the rate of disappearance of the peroxide. To study the action of amylase upon starch, we should take solutions of starch and of amylase of known concentrations, keep them in a water bath at 38° C., and at a certain point add some enzyme solution to the starch solution. At periods of five or ten minutes after the addition had been made 5 c.c. of the mixture might be withdrawn for determination of the amount of sugar formed by the action of the enzyme. After obtaining a series of data in this way, a curve could be drawn, showing the amount of change of starch which had occurred in each unit of time. In the case of the action of sucrase on cane sugar the investigation is still easier. Since the change from cane sugar to invert sugar is accompanied by a change in the rotatory power of the solution on polarised light, it is necessary only to put the mixture of enzyme and cane sugar into a polarimeter tube, which is kept at a constant temperature by means of a water jacket, and read off at intervals of a few minutes the change in the rotatory power of the solution. From this change can be easily calculated the percentage of cane sugar which has been converted into fructose and glucose.

In investigating the action of proteolytic enzymes, as *e.g.* that of trypsin on caseinogen, samples are taken at intervals and subjected to proper analytical procedures to determine the amino-groups set free (liberation of N_2 on treatment with nitrous acid—Van Slyke*), or carboxyl groups liberated (by titration in alcoholic solution—Willstatter and Waldschmidt Leitz†). These give an indication of the number of peptide linkages which have been split, *i.e.* of new molecules formed. A very convenient method is that employed by Henri and by Bayliss‡ in the investigation of the kinetics of tryptic action, namely the determination of the electrical conductivity of the solution. In the disintegration of the molecule caused by the enzyme, there is a continuous increase

* Van Slyke. *J. Biol. Chem.*, 1929, **83**, 425.

† Wilstatter and Waldschmidt Leitz. *Ber. d. deutsch. Chem. Gesselsch.*, 1921, **54**, 2988; Wilstatter. *Abderhalden's Hdbch. d. biol. Arbeitsmeth.*, 1922, Abt. I. Teil 7, 298; Richardson. *Proc. Roy. Soc.*, 1934, **115B**, 121, 142.

‡ W .M. Bayliss. *Arch. des Sci. Biol.*, 1904, **11**, Supt. 261.

in the conductivity of the solution, and this increase can be regarded as an index to the rate of change in the substances undergoing disintegration.

By such methods it has been found in the simplest case, when the substrate is in true solution, that, when the quantity of enzyme employed is very small in comparison with the *substrate* (the substance acted upon), the *amount of change in a given time is proportional to the amount of enzyme* present, and is (within limits) independent of the concentration of the substrate. This is well shown by the two following Tables representing the action of lactase upon lactose (E. F. Armstrong *):

PROPORTIONS HYDROLYSED IN 100 C.C. OF A 5 PER CENT. SOLUTION OF LACTOSE. ENZYME VARIED.

Solutions containing—	15 hours	20 hours	45 hours
1 c.c. lactase . . .	0·15	2·2	3·9
10 c.c. „ . . .	1·6	23·3	38·6
20 c.c. „ . . .	3·2	45·8	—

LACTOSE HYDROLYSED. ENZYME CONSTANT, SUBSTRATE VARIED.

Solutions containing—	24 hours		46 hours	
	Proportion	Weight	Proportion	Weight
10 per cent. lactose .	14·2	1·42	22·2	2·22
20 „ „ .	7·0	1·40	10·9	2·18
30 „ „ .	4·8	1·44	7·7	2·21

Moreover if we take only the earlier stages of the enzyme action, it is found that, with small proportions of enzyme, equal amounts of substrate are changed in successive intervals of time until about 10 per cent. has been hydrolysed. This is shown in the following Table:

2 PER CENT. LACTOSE WITH LACTASE

Time	Amount hydrolysed
⅓ hour	3·2
⅔ „	6·4
1 „	9·6
2 hours	16·4
3 „	20·8

These results can best be interpreted by assuming that the first stage in the reaction is a combination of enzyme with substrate. Bayliss believed the combination to be an adsorption, whereas Michaelis thought it to be a reversible combination of the ordinary chemical equilibrium type. In either case, it is the concentration of this combination which represents the active mass of the molecules, *i.e.* the molecules of substrate which are undergoing change. This compound, as soon as it is formed, takes up water and breaks down, setting free the hydrolysed substrate and the enzyme, which is at once ready to combine with a further portion of the substrate. In such a case the velocity of reaction must be directly proportional to the amount of enzyme, and the same absolute quantity of substance will continue to be changed in succeeding units of time. Supposing, for instance, we had at the bottom of a hill a load of bricks which had to be transferred to the top, and five men to effect the transference. The rate of transference would be directly proportional to the number of men employed; we could double the rate by

* E. F. ARMSTRONG. *Proc. Roy. Soc.*, 1904, **73**, 500.

doubling the men. Moreover the number of bricks carried in each unit of time would be the same. Five men would carry as many bricks in the second ten minutes as they would in the first, and so on. On the other hand, the velocity with which the transference was effected would be independent of the number—that is, the *concentration*—of the bricks at the bottom of the hill. The *active mass* of bricks could be regarded as that number carried at any moment by the transferring factor, namely the men. The equation of change would be $\phi = KC$, where C is the concentration of the enzyme. This concentration is always being renewed and kept constant by the breaking down of the intermediate product, so that the rate of change would be continuous throughout the experiment.

On the other hand when the amount of enzyme is relatively large, the rate of change, though at first very rapid, tends continuously to diminish. This is shown by the following Table representing the rates of change, during succeeding intervals of ten minutes, in a caseinogen solution to which a strong solution of trypsin had been added (Bayliss).

VELOCITY OF TRYPSIN REACTION

6 c.c. 8 per cent. Caseinogen + 2 c.c. $\frac{N}{10}$ NH_4OH + 2 c.c. 2 per cent. Trypsin at 39° C.

1st 10 minutes	K = 0 0079
2nd ,,	0 0046
3rd ,,	0 0032
4th ,,	0 0022
5th ,,	0 0016
6th ,,	0 0009
&c.	&c.

In this retardation of the later stages of enzyme action, the end products are concerned in some way or other, and the retardation can be augmented by adding to the digesting mixture the boiled end products of a previous digestion. The retarding effect of the end products resembles in many ways that observed in reversible reactions, and is most simply regarded as being due to the fact that the enzyme reversibly combines not only with the substrate, but also with the end products, tending to reverse the reaction, or to prevent enzyme from combining with substrate, or both.

In general, however, reversal of enzyme actions is not easily attained, so that probably a more important factor in slowing the enzyme action in its final stages is the combination of the enzyme itself with the end products and the consequent removal of the enzyme from the sphere of action. Several facts speak for such a mode of explanation. Thus the action of lactase on milk sugar is not retarded by both its end products glucose and galactose but only by galactose. In the same way the action of invertase on cane sugar is retarded by the end product fructose but not by glucose.

So far, therefore, a study of the velocity of enzyme actions would lead us to suspect that the enzyme in the first place combines with the substrate, and that this combination is a necessary step in the alteration of the substrate. In the second place, the enzyme is taken up to a certain extent by some or all of the end products, and this combination acts in opposition to the first combination, tending to remove the enzyme from the sphere of action and therefore to retard or even reverse the whole reaction.

ACTIVATION OF ENZYMES. Many enzymes are quite inactive unless some other substance or group of substances is present with the enzyme. Thus the amylase of saliva is inactive in absence of chloride or bromide ions, and phosphatase in absence of magnesium ions.

Some enzymes when set free into secretions, or when extracted from cells, are nearly inactive unless certain specific " activators " are also present. Thus trypsin of the pancreatic juice requires the presence of enterokinase before it will act on most native proteins, though the trypsin alone is capable of hydrolysing protamines or peptones. Enterokinase is a specific *kinase*, and like other kinases, is colloidal.

Another type of activation is seen with lipases : that of the pancreatic juice, for instance, is rendered much more active by the presence of bile salts, albumin or calcium salts. This phenomenon is due to the fact that the fat, lipase and protein are adsorbed together and thereby brought more closely into contact with one another, and with water.

All enzymes are very sensitive to changes of hydrogen ion concentration, and there is an optimum *p*H at which each enzyme is most active, *e.g.* for pepsin *p*H 1·5, for trypsin *p*H 8·3, &c. The optimal *p*H, however, depends very much on other conditions, *e g.* in the presence of impurities it may be considerably altered.

CO-ENZYMES. Certain types of enzymes, concerned in oxidation—reduction processes, require the presence of specific catalysers which are called co-enzymes. They are organic substances, often nucleotides containing phosphate groups, and are heat stable and dialysable.

In some instances the co-enzyme is a complex mixture and is called a *co-enzyme system.* For example, muscle contains an enzyme which can convert glycogen to lactic acid, provided there be present a co-enzyme system consisting of cozymase, adenyl pyrophosphate, a phosphate, and magnesium. Enzymes may be freed from their co-enzymes by dialysis, *i.e.*, the co-enzymes are not colloids.

ENZYMES AS SYNTHETIC AGENTS

Many enzymes have been found capable of catalysing reactions in the reverse direction and forming the original substrate, or a substance closely resembling it. This happens most readily if the concentration of normal end products is very great. Croft Hill * showed that if maltase were added to a 40 per cent. solution of glucose, a change took place in the reverse direction, which proceeded until 85 per cent. of the glucose was left. The sugar formed, a disaccharide, is not maltose, but the stereoisomeric sugar, iso-maltose.

In the same way it was found by Castle and Loewenhart that the hydrolysis of esters by lipase is a reversible reaction, the action of lipase being simply to hasten the attainment of the equilibrium point between the four substances—ester (or neutral fat), water, fatty acid and alcohol. It has been shown † that " plastein " resembling a protein can be resynthesised from protein hydrolysis products by the action of pepsin at a *p*H of 4·0 and at a relatively high temperature, *e.g.* 70° C. Addition of protein delays the synthesis. A similar synthesis is effected by trypsin at *p*H 5·7.

If all enzyme actions are in this way reversible, a possibility is opened of regarding the synthetic processes occurring in the living cell, as well as the processes of disintegration, as determined by the action of enzymes. It must be noted that these effects are obtained with distinctness only when dealing with concentrated solutions. The degree of synthesis, which would be produced in the very dilute solutions of glucose, &c., occurring in the animal cell, would therefore be infinitesimal. But if a mechanism were provided for the immediate separation of the synthetic product from the sphere of reaction, either by removing it to a different part of the cell or by building it up into some more complex body which was not acted on by the enzyme, the process of synthesis might go on indefinitely, and the infinitesimal quantities be summated to an appreciable amount.

* CROFT HILL. *Trans. Chem. Soc.*, 1898, **73**, 634.

† BORSOOK, MACFADYEAN and WASTENEYS. *J. Gen. Physiol.*, 1930, **13**, 295.

THE NATURE OF ENZYMES. By the use of selective adsorption, enzymes may be separated from one another and from many accompanying impurities, and obtained in a state of great concentration. Crystalline preparations of several enzymes, *e.g.* urease *, pepsin †, chymotrypsin, trypsin ‡, amylase, etc., have been obtained.

All enzymes are colloidal, and a theory (Willstätter's) much in favour suggests that an enzyme consists of two closely associated substances or groups, a colloidal "carrier" which is non-specific, and one or more specific active groups, by which union with the substrate is effected. This union takes place with definite groups in the substrate molecule, and the specific action of the enzyme is determined by the nature and position of the receptor group to which the specific group of the side chain becomes attached. Thus, in the enzymes of the peptidase class the imino group is one point of attachment. The purest enzymes, crystalline pepsin §, trypsin ||, urease ¶ and amylase ** are proteins, and probably all enzymes are of protein nature, or contain a protein as an essential constituent. Some (*e.g.* lipase, phosphatase) depend for their activity on possession of an SH group, while others do not. Many enzymes can exist in an inactive form which is convertible, by slight molecular rearrangement, into the active form, the conversion in some cases, *e.g.* in the activation of trypsin, being autocatalytic, *i.e.* it is started by a minute trace of the active form, and proceeds with rapidly augmenting velocity as the active form increases in amount.††

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TYPES OF CHEMICAL CHANGE

In spite of the enormous diversity of chemical reactions occurring in the body, they may be divided into a relatively small number of types. Nearly all the reactions are reversible. The chief types of chemical change are as follows :

(1) HYDROLYSIS. In most cases this involves the taking up of water and a decomposition into smaller molecules. Thus, the proteins are broken down by a series of proteases into their constituent amino-acids. The disaccharides, such as maltose or lactose, under the influence of the appropriate carbohydrases, take up one molecule of water and give rise to two molecules of monosaccharide. The fats, under the action of lipases, take up three molecules of water with the formation of fatty acid and glycerol. Hippuric acid is broken down into benzoic acid and glycine by histozyme, and urea to ammonia and carbon dioxide by urease. The enzyme glyoxalase converts pyruvic aldehyde to lactic acid.

The reverse change, that of dehydration, is also effected apparently with equal facility by the living cell, the hexoses losing water and being converted into a complex starch or glycogen molecule, while the amino-acids are built up, first into polypeptides, and these again into the complex proteins of the cell.

Besides the reactions in which there is a difference in the amount of free water on the two sides of the equation, it seems probable that hydrolysis and simultaneous dehydration at different parts of the molecule determine

* SUMNER. *J. Biol. Chem.*, 1926, **69**, 435 ; **70**, 97.
† NORTHROP. *J. Gen. Physiol.*, 1931, **14**, 713.
‡ NORTHROP and KUNITZ. *J. Gen. Physiol.*, 1932, **16**, 267.
§ NORTHROP. *J. Gen. Physiol.*, 1930, **13**, 739.
|| NORTHROP and KUNITZ. *J. Gen. Physiol.*, 1932, **16**, 267.
¶ SUMNER, KIRK and HOWELL. *J. Biol. Chem.*, 1932, **98**, 543.
** CALDWELL and SHERMAN. *Science*, 1931, **74**, 37.
†† NORTHROP. *Science*, 1937, **86**, 479.

a number of chemical transformations, which at first sight seem to involve a simple splitting of the molecule.

(2) DEAMINATION. This process involves the splitting off from an amino-acid of an NH_2 group as ammonia. Many tissue of the body appear to have this power, though little is known of the enzymes by which the change is affected. If, for instance, to slices of surviving liver or kidney, an amino-acid be added, ammonia is set free in proportion to the amount of amino-acid added.

Although the simplest mode of deamination of an amino-acid would apparently be the hydrolytic one, with formation of ammonia and a hydroxy acid, work by Neubauer showed that deamination is accompanied in the first place by oxidation, so that the first intermediate product formed is an α-ketonic acid.

$$\begin{array}{l} CH_3 \\ | \\ CHNH_2 \\ | \\ COOH \end{array} + O = \begin{array}{l} CH_3 \\ | \\ CO \\ | \\ COOH \end{array} + NH_3$$

(Alanine) (Pyruvic acid)

Knoop showed that the reverse change may occur. On administering benzyl-pyruvic acid ($C_6H_5.CH_2.CH_2.CO.COOH$) to a dog, a certain amount of benzylalanine ($C_6H_5.CH_2CH_2CHNH_2.COOH$) appeared in the urine.

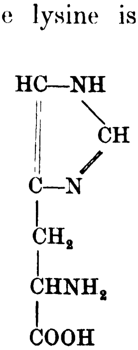
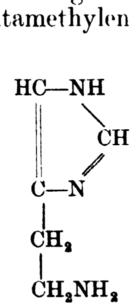
(3) DECARBOXYLATION. This involves the removal of a carboxyl group, with formation of CO_2, and is probably one way in which that end-product is set free. In the formation of alcohol from glucose by yeast, pyruvic acid is formed at one stage, and is then acted upon by an enzyme, *decarboxylase*, of the yeast, forming acetaldehydde and CO_2 :—

$$CH_3.CO.COOH = CH_3CHO. + CO_2$$

The enzyme needs a co-enzyme, called co-carboxylase, in order to function. It has been shown that co-carboxylase is a pyrophosphoric ester of vitamin B_1 (v. p. 159).

Many amino-acids when subjected to the action of bacteria lose a molecule of carbon dioxide and are converted into a corresponding amine.

For instance lysine is converted into pentamethylene diamine or cadaverine ;

and Histidine  becomes Histamine 

In the same way ornithine is converted into tetra-methylene diamine or putrescine. Other examples of this process of decarboxylation are :

Isoamylamine $(CH_3)_2.CH.CH_2.CH_2.NH_2$, from leucine.
β-phenylethylamine $C_6H_5.CH_2.CH_2.NH_2$, from phenylalanine.
Para-hydroxyphenylethylamine $HO.C_6H_4.CH_2.CH_2.NH_2$, from tyrosine.

A similar process has been supposed to take place as a step in the successive oxidation of the carbon atoms in the long chain fatty acids or carbohydrates.

It is worthy of note that all the changes which we have been considering involve but little expenditure of energy. This is evident if we examine the heat evolved by the total combustion of 1 gramme molecule of the initial and final substances in the three classes of chemical change that we have just been considering :

(1) HYDROLYSIS

Initial substance	Heat of combustion Cal per molecule	Final substance	Heat of combustion
Maltose	1350	2 Glucose	1354
Glucose	677	2 Lactic acid	659
Hippuric acid	1013	Glycine / Benzoic acid	235 / 773 } 1008

(2) DEAMINATION

Initial substance	Heat of combustion	Final substance	Heat of combustion
Alanine	389·2	Lactic acid	329·5
Leucine	855	Caproic acid	837
Aspartic acid	386	Succinic acid	354

(3) DECARBOXYLATION

Initial substance	Heat of combustion	Final substance	Heat of combustion
Alanine	389	Ethylamine	409
Leucine	855	Isoamylamine	867

(4) OXIDATION AND REDUCTION. The fourth class of chemical reactions differs from those just described in being attended with a very considerable energy change. This class involves the processes of oxidation and reduction. In almost every living cell, by far the largest amount of the energy available for the discharge of the functions of the cell is derived from the oxidation of the foodstuffs. If we compare the initial with the final products in the oxidation of the foodstuffs, the very large evolution of energy is evident. Thus in the conversion of glucose into CO_2 and water there is an evolution for each gramme molecule of 677 Calories. The change does not in the living cell occur all at once, but the molecule is oxidised step by step. The energy thus set free may be liberated as heat or converted into work, mechanical or osmotic, or it may be used in whole or in part for effecting other chemical reactions which involve absorption of energy.

The oxidation of an organic substance may either be an actual addition of oxygen to its molecule, as when an aldehyde is converted to an acid :

$$R.CHO \longrightarrow R.COOH$$

or a withdrawal of hydrogen from the molecule, as in the oxidation of an alcohol to an aldehyde :

$$R.CH_2OH \longrightarrow R.CHO.$$

Whichever process happens, it is important to remember that *oxidation is always accompanied by reduction* of some other substance, *e.g.* by loss of oxygen or by gain of hydrogen. In the former case the substance simultaneously reduced is called an *oxygen donator* and the substance oxidised an *oxygen acceptor* ; in the latter case the substance reduced is a *hydrogen acceptor*, the oxidised substance being a *hydrogen donator*.

In some instances a molecule of water is involved, and its hydrogen reduces one molecule of a substance, while its oxygen oxidises another mole-

cule of the same substance. This change is effected by the enzymes, called *mutases*, and the change is known as *dismutation*.* It is exemplified by the conversion of two molecules of an aldehyde into one of alcohol and one of acid :—

$$\begin{matrix} R.CHO \\ + \\ R.CHO \end{matrix} + H_2O = \begin{matrix} R.CH_2OH \\ + \\ R.COOH \end{matrix}$$

Here one molecule is hydrogen acceptor, the other oxygen acceptor.

Most of the foodstuffs, and the constituents of the organism which undergo oxidation in the body, are comparatively stable substances with little affinity for free oxygen at body temperature. Free oxygen and hydrogen, similarly, do not readily combine at ordinary temperatures. The question arises as to the means available in the body for bringing either free oxygen or the hydrogen of the substance into such a state of activation that the oxygen can act as an oxygen donator or hydrogen acceptor. This is effected indirectly by the agency of oxidising enzymes, of which there are two types, one dependent upon the activation of oxygen, the *aerobic oxidases ;* the other the *dehydrogenases*, independent of free oxygen, but activating hydrogen in the oxidisable compound, this hydrogen then being transferred to a reducible substance.

Biological oxidations are all due ultimately to a transfer of electrons from the substance to be oxidised, first to an oxidising catalyst, and then to molecular oxygen. The oxidising catalysts, or enzymes, usually consist of two parts, one a protein and one a reversible oxidation—reduction system combined with the protein part.

Aerobic Oxidases. It has long been known that hydrogen peroxide is able to effect oxidative changes similar to those observed in the animal body. Thus Dakin showed that the series of fatty acids, under the action of hydrogen peroxide, undergoes progressive oxidation in the β position, so that the carbon atoms are removed from the long chain of fatty acids two at a time. The action of hydrogen peroxide is much quickened by the presence of a trace of iron in the ferrous condition (Fenton's reaction) : the iron thus acts as a catalyser.

There is evidence that a similar system, peroxide + catalyser, is sometimes employed in the living cells. According to Bach and Chodat, many of the oxidations occurring in organisms are effected by the simultaneous action of an *peroxidase* and of a *oxygenase.* The peroxidase is an enzyme. In some cases it may be associated with an iron compound. It can be poisoned by HCN or CO. Peroxidases are more readily demonstrable in plant than in animal tissues. They are detected by the fact that they oxidise certain substances to form coloured products. Thus guaiacum resin or guaiaconic acid is turned blue *in presence of a peroxide and a peroxidase ;* if the enzyme preparation also contains an oxygenase then it turns guaiacum blue without addition of a peroxide. The oxygenase is an organic substance which is spontaneously oxidised by free oxygen to form a peroxide, either an organic one or hydrogen peroxide. The peroxidase is able to produce active oxygen in presence of these peroxides, so that the mixture of oxygenase and peroxidase, which is called an *aerobic oxidase*, is able to carry out oxidations *when free oxygen is present.* An important animal peroxidase is tyrosinase, which oxidises tyrosine to a black pigment, melanin, in presence of oxygen. Another is indophenol oxidase,

* GREEN, *et al. Bioch. J.*, 1937, **31**, 2327.

which in presence of p-phenylene diamine and α-naphthol forms a blue compound.

Some plant oxidases contain copper and are also inhibited by KCN, H_2S and CO.*

The almost universal presence of a *catalase* in tissues serves to destroy any excess of hydrogen peroxide which is produced in this way. Catalase, a methæmoglobin-like substance, breaks up hydrogen peroxide with liberation of molecular oxygen; it cannot function as a peroxidase, which splits off oxygen from hydrogen peroxide in the active condition.

Keilin † has shown that iron-containing substances, the *cytochromes*, which are widely distributed in all tissues that use oxygen, play an important part in the process of oxidation by taking up free oxygen which is then passed on to oxidisable substances in the tissues. The pigments can occur in an oxidised and a reduced form, and in presence of oxygen are converted to the former by the action of a variety of indophenol oxidase, called cytochrome oxidase, and then reduced by dehydrogenases as described below. There are several cytochromes, all of them hæmatin-like compounds, and the cytochrome oxidase is another similar substance forming part of the "cytochrome system." ‡ This system acts as a middleman, conveying oxygen from the air to take up the hydrogen activated by the action of dehydrogenases. Oxidation of the cytochrome of the tissues is prevented by traces of HCN, CO, or sulphides because of their toxic action on the peroxidase. Reduction of the oxidised cytochrome is inhibited by urethane, or alcohol, which block the action of the dehydrogenase.

Dehydrogenases. We have seen that oxidation may imply withdrawal of hydrogen instead of the addition of oxygen to a molecule. Such an oxidation could take place in absence of free oxygen, but in that case, if the process is to continue, there must be some other substance present which can act as a hydrogen acceptor, taking up the hydrogen which has been set free by the reaction. Thus we should expect processes both of oxidation and reduction to be continually occurring in the living tissues, though the final result will be one in which oxidation predominates. That both these processes occur is shown by the behaviour of methylene blue in contact with the tissues or with extracts of the tissues. If washed minced muscle be placed in a dilute solution of methylene blue, a little succinic acid added to it, and the mixture placed out of contact with oxygen, and kept warm, the methylene blue is quickly reduced to a colourless compound, and an equivalent amount of succinic acid is oxidised to fumaric acid. The reaction is one of hydrogen transfer, the succinic acid losing two atoms:

$$C_4H_6O_4 \longrightarrow C_4H_4O_4 + 2H,$$

and the 2H is taken up by the methylene blue. This is an oxidation independent of free oxygen and is effected by an enzyme called a *dehydrogenase*. On shaking the mixture with air, the colour is restored owing to the reformation of methylene blue. In these processes of reduction and oxidation in tissues many different enzymes are involved, according to the nature of the tissue and the nature of the substance, *e.g.* fat, amino-acid or carbohydrate, which is undergoing oxidation.

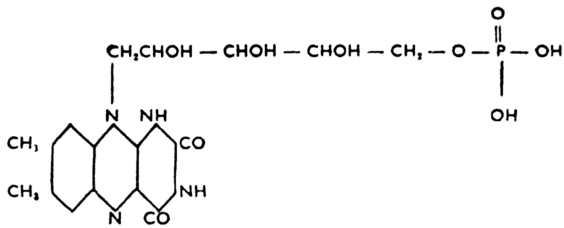
An important oxidation system present in cells is that containing the so-called *yellow oxidising enzyme.*§ This consists of a phosphorylated flavine plus a protein, the flavin being identical with riboflavine, or vitamin B_2.

* KEILIN and MANN. *Proc. R. S. B.*, 1938, **125**, 187

† KEILIN. *Proc. Roy. Soc.*, 1929, **104B**, 206.

‡ KEILIN and HARTREE. *Proc. R S*, 1939, **127B**, 167.

§ WARBURG and CHRISTIAN. *Bioch. Ztschr.*, 1933, **266**, 405; 1938, **298**, 150; THEORELL, *Bioch. Ztschr.*, 1935, **278**, 263.



PHOSPHORYLATED RIBOFLAVINE. (+ *protein = yellow enzyme of Warburg*).

It is now believed that the substance which actually occurs in cells in combination with protein is an alloxazine-adenine-dinucleotide, of which the yellow enzyme is a decomposition product. The method of action is the same, but the dinucleotide is much more active.

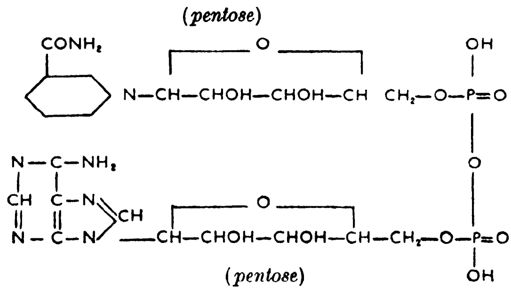
Like cytochrome, these substances can undergo reversible oxidation and reduction, and so act as oxygen carriers.

The yellow enzyme, or flavoprotein, acts as part of a double enzyme system, the other part being an activator consisting of an "*intermediary*" ferment, which consists of a protein (present in all cells and usually obtained from blood) plus a co-enzyme.

This co-enzyme, also called *co-zymase*, is a compound of nicotinic amide, adenine, two molecules of pentose, and one of pyrophosphoric acid, and has the probable formula * :—

(*nicotinic acid amide*)

(*adenine*)



CO-ZYMASE

The whole system can *e.g.*, oxidise hexose-monophosphate to an acid, with absorption of oxygen, and this happens in several stages.† First, the co-enzyme (Ko) in presence of the intermediary ferment brings about the oxidation of the hexose- mono-phosphoric acid :—

$$\mathrm{Ko} + \underset{\text{(Hexose-monophosphoric acid)}}{\mathrm{R.COH}} + \mathrm{H_2O} = \mathrm{Ko}\begin{matrix} \diagup \mathrm{H} \\ \diagdown \mathrm{H} \end{matrix} + \underset{\text{(Phosphohexonic acid)}}{\mathrm{RCOOH}} \quad \ldots \ldots \quad (1)$$

being itself reduced.

Next, the yellow enzyme complex (Y) is reduced and the co-enzyme system re-oxidised by transfer of hydrogen :—

$$\mathrm{Y} + \mathrm{Ko}\begin{matrix} \diagup \mathrm{H} \\ \diagdown \mathrm{H} \end{matrix} = \mathrm{Y}\begin{matrix} \diagup \mathrm{H} \\ \diagdown \mathrm{H} \end{matrix} + \mathrm{Ko} \quad \ldots \ldots \quad (2)$$

and lastly, the reduced yellow ferment, being autoxidisable, is re-oxidised by free oxygen, hydrogen peroxide being a bye-product :—

$$\mathrm{Y}\begin{matrix} \diagup \mathrm{H} \\ \diagdown \mathrm{H} \end{matrix} + \mathrm{O_2} = \mathrm{H_2O_2} + \mathrm{Y} \quad \ldots \ldots \quad (3)$$

This enzyme system can act in absence of all iron compounds and in presence of cyanides, but when cytochrome is present probably plays only a small part in cellular oxidations.

* EULER and SCHLENK. *Z. f. physiol. Chem* , 1937, **246**, 64.
† WARBURG, CHRISTIAN and GRIESE. *Bioch. Ztschr.*, 1935, **282**, 157.

Another important substance is *glutathione* *, which is almost universally present in animal tissues. It is a tripeptide of glycine, cystein and glutamic acid, and is γ-glutamyl-cysteyl glycine †. Its structural formula is :

$$\underset{\text{Glutamyl}}{H_2N.\overset{\overset{\textstyle COOH}{|}}{C}H.CH_2.CH_2.CO}\,\underset{\text{cysteyl}}{NH.\overset{\overset{\textstyle HSCH_2}{|}}{C}H.CO}\,\underset{\text{glycine}}{NH.CH_2COOH}$$

Although present in minute quantities, it plays an important part in the processes of oxidation and reduction which occur in the cells, since under suitable conditions it can function either as a hydrogen acceptor or as an oxygen acceptor, as seen from the following equation :

$$2\cdot GSH \underset{H_2}{\overset{O}{\rightleftharpoons}} GSSG$$

G stands for the large part of the molecule, which is not directly concerned in these changes. In the reduced form this compound, when in neutral or slightly alkaline solution, readily undergoes oxidation by air. It therefore acts as an oxygen acceptor, the oxygen being converted into hydrogen peroxide, so that it functions as an oxygenase. On the other hand, the oxidised or di-sulphide form is reduced by the reducing substances in tissues when on the acid side of neutrality. Thus the rate of reduction of methylene blue by fresh tissues is retarded in acid solution by the presence of the disulphide form, because this competes with the methylene blue for hydrogen ; whereas in neutral or alkaline solutions the presence of the disulphide accelerates decolorisation of the blue, because as soon as some of it is reduced it becomes autoxidised back again.

Freshly minced muscular tissue respires, *i.e.* it takes up oxygen and gives off CO_2. If it be thoroughly washed, this process of respiration, as well as its property of reducing methylene blue, comes to an end. This addition of glutathione to the washed muscle restores not only the respiration but also the power of reducing the dyestuff. Meyerhof has shown that, even if the muscle tissue has been heated to 100° C., the addition of glutathione to it still restores the property of ' respiration ' and of reducing methylene blue. It seems that it is the unsaturated fatty acids, contained in the lecithin of the washed and heated muscle, and the proteins, which undergo oxidation in the presence of air and glutathione. Thus in addition to those mechanisms for oxidation and reduction which are thermolabile, there are present in the cell other systems such as the glutathione system, and part of the cytochrome, which can resist the temperature of boiling water. Another important function of glutathione appears to be in acting as a co-enzyme for glyoxalase.‡ This enzyme converts methyl glyoxal intol actic acid §, and in this reaction glutathione is a catalyst, which appears to function as follows || :—

$$\underset{\text{(methyl glyoxal)}}{CH_3.CO.CHO} + \underset{\substack{\text{(reduced}\\\text{glutathione)}}}{G.SH.} \rightleftharpoons$$

$$\underset{\substack{\text{(intermediate compound with}\\\text{oxidised glutathione)}}}{CH_3.CO.CHOH.S.G} \xrightarrow{+H_2O} \underset{\text{(lactic acid)}}{CH_3.CHOH.COOH} + \underset{\substack{\text{(reduced}\\\text{glutathione)}}}{G.SH.}$$

In the oxidation of lactic acid, which plays so important a part in the activity of muscle and other tissues, it is possible that a special dehydrase, the lactic dehydrase ¶, which acts similarly to the succinic dehydrase described above, oxidises the lactic acid to pyruvic acid.

Another substance capable of alternate oxidation and reduction in living cells, and which may play a part as an oxygen carrier, is *ascorbic acid* (or vitamin C).

* HOPKINS. *Bioch. J.*, 1921, **15**, 286 ; *J. Biol. Chem.*, 1929, **84**, 269 ; *Proc. Roy. Soc.*, 1931, **109B**, 58.

† PIRIE and PINHEY, *J. Biol. Chem.*, 1929, **84**, 321 ; HARINGTON and MEAD, *Bioch. J.*, 1935, **29**, 1602 (Synthesis).

‡ LOHMANN. *Bioch. Z.*, 1932, **254**, 332.

§ DAKIN and DUDLEY. *J. Biol. Chem.*, 1913, **14**, 155.

|| JOWETT and QUASTEL. *Bioch. J.*, 1933, **27**, 486.

¶ BERNHEIM. *Biochem. Journ.*, 1928, **22**, 1178.

In most cases the oxidative processes in the cell depend on the integrity of the structure of the cell, so that they are much diminished if the cell structure is destroyed by grinding. We are probably concerned here with surface effects occurring at the interfaces between different phases of the cell protoplasm and especially at the surfaces of the cell itself and of the nucleus. Similar effects of surface are familiar in non-living material Thus certain amino-acids, especially cystein, are oxidised at room temperature to carbon dioxide and ammonia on shaking their solution with blood charcoal. This oxidation probably takes place at the surface of the charcoal particles, and it seems that here the presence of iron plays an important part as a surface catalyser. It is interesting to note that the oxidation of amino-acids by charcoal is inhibited, in a manner similar to inhibition of oxidation in the living cell, by narcotics and by cyanides. Warburg suggests that narcotics act by being adsorbed on the surface and thus displacing the oxidisable substances, whereas cyanides probably act by inactivating the iron catalyst.

Oxidation-reduction Potentials. Although for most purposes it is sufficient to consider the phenomena of oxidation and reduction as simple chemical reactions, it is often valuable to regard them from the physico-chemical standpoint, as questions of transfer of electrons. In this sense oxidation is regarded as the loss of an electron, or of electrons, from an atom, and reduction as a gain of electrons. Thus when ferrous iron is oxidised to ferric iron an electron or charge of negative electricity is lost, so that the ferric iron atom gains a positive charge. This is expressed by the electro-chemical equation :—

$$Fe^{++} \rightleftarrows Fe^{+++} + e,$$

the quantum e for 1 gramme-atom being 1 Faraday, or 96,500 coulombs of negative electricity. It will be seen that this conception is really more accurate than one involving a transfer of either hydrogen or oxygen, since it covers all cases, *e.g.* the oxidation of ferrous chloride to ferric chloride, as above. Oxidation is always accompanied by reduction in some other substance, which thereby gains the electron lost by the oxidised substance. In cases where there is merely a transfer of electrons in this manner there is of course no energy change, but often there is liberation of some of the energy as heat or in some other form.

In order for oxidation to occur, there must be a readiness for the electron to escape, and the greater its escaping tendency, or *fugacity*, the more readily will oxidation take place. This tendency can be measured for any dissolved substance by the determination of the *oxidation-reduction potential*, which may be explained as follows. Suppose that into a readily oxidisable solution, *e.g.* $FeCl_2$, we dip a clean gold or platinum surface, then the electrons, by their tendency to escape, will give to this a negative charge, which will be greater the more the electron-fugacity of the substance. We thus have a half-cell, which may be combined with another half-cell, *e.g.* a hydrogen electrode, very much as described on p. 69, to form a cell the potential of which can be measured by some method which will not draw much current, *e.g.* an electrometer. The negativity of the gold electrode, as thus measured, is a measure of the ease of oxidisability of the substance into which it is dipped, or alternatively, of the reducing power of that substance towards other substances. Conversely, the less negative (or more positive) the electrode the greater the reducibility (or oxidising power) of the substance.

It is a convention to express the results in terms of what is called E_h, which is the reading, in volts, of the oxidation-reduction cell when set up with a normal hydrogen electrode, *i.e.* N.HCl. It follows from what has been said above that a solution of a substance capable of *reversible* oxidation and reduction will oxidise substances whose solutions have a more negative E_h (*i.e.* it will take up electrons from them), and reduce substances which have a more positive E_h than its own (*i.e.* it will give up electrons to them).

If we take a mixture of such a substance in the oxidised and reduced forms, it will have a more negative E_h the more of the reduced form it contains, and *vice versâ*. Many dyes are capable of oxidation and reduction, and since the reduced forms are often colourless, such dyes, when added to mixtures of other substances, become oxidised

or reduced according to the oxidising or reducing potentials of the whole system in which they are present. We can use these dyes to assess the oxidation-reduction potentials in a manner somewhat analogous to the use of coloured buffer-dyes for the assessment of the hydrogen ion concentration of a mixture.

It has long been known that dyes such as methylene blue can often be reduced by most tissues; others, such as indophenol blue,* are reduced by some tissues but not by others, such as heart or brain: alizarin blue has so high a reduction potential that it could only be reduced by a few organs, *e.g.* lung, liver. Further, we know that such reversibly oxidisable substances, by being alternately reduced and oxidised, can act as hydrogen or oxygen carriers, and thus act as oxidation or reduction catalysts. Many naturally occurring substances, both in plants and animals, have this property, *e.g.* glutathione, ascorbic acid, various flavins, &c. Such substances, loosely linked to protein, form the oxidising enzymes, and "when a group of these enzymes and their oxidisable substances act together . . . the oxidation reactions proceed, as a rule, in a series, the system of more negative potential being oxidised by the reduction of the system of more positive potential, and so on, in a series of graded steps up to the reduction of molecular oxygen. So, like locks in a canal, these reversible systems release gradually the oxidation energy" (Barron).

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* EHRLICH. "Das Sauerstoffbedürfniss des Organismus," Berlin, 1885.

BOOK II

MUSCLE AND NERVE

CHAPTER X

VOLUNTARY STRIATED MUSCLE

THE movements of the muscles are normally carried out in response to changes aroused in the central nervous system by events occurring in the environment of the body. Every movement of an animal is thus in its most primitive form a reflex action, and involves changes in a peripheral sense organ, in an afferent nerve fibre, in the central nervous system, and in an efferent nerve fibre, before the actual process of contraction occurs in the muscle itself and gives rise to the resultant movement (Fig. 33). If we are to determine the nature of the changes involved in this reflex action, we must be able to study them as they progress along the different elements which make up the *reflex arc*. This analysis is facilitated by the fact that we are able to arouse a condition of activity in the different parts

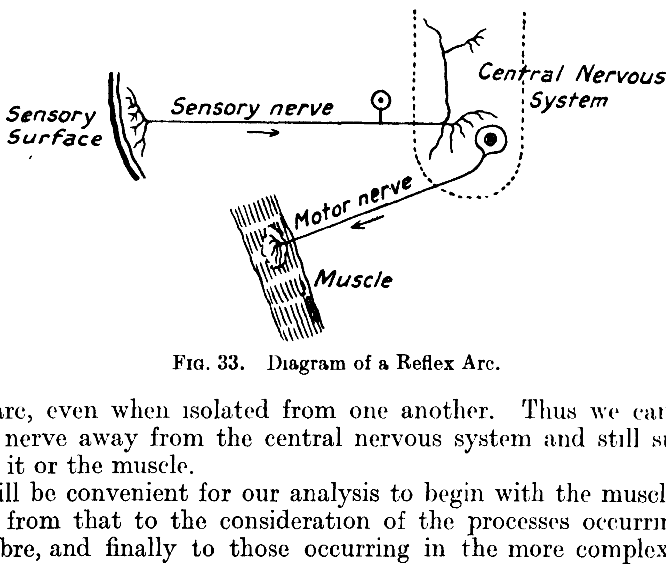

FIG. 33. Diagram of a Reflex Arc.

of the arc, even when isolated from one another. Thus we can cut the efferent nerve away from the central nervous system and still succeed in exciting it or the muscle.

It will be convenient for our analysis to begin with the muscle, and to proceed from that to the consideration of the processes occurring in the nerve fibre, and finally to those occurring in the more complex nervous system.

In the higher animals we may distinguish three varieties of muscle. All movements that require to be sharply and forcibly carried out are effected by means of striated muscular tissue and, as these movements are in nearly all cases under the control of the will, the muscles are generally spoken of as *voluntary*. Plain or *involuntary* muscles form sheets or closed tubes surrounding the viscera, the blood vessels, &c. By their slow prolonged contractions they serve to maintain and regulate the flow of the contents of these organs. Intermediate in properties as well as in structure between these two classes is the *heart muscle*. This, though striated, presents considerable variations both in structure and function from ordinary skeletal muscle.

The properties of contractile tissues have been most fully investigated in the voluntary muscles, almost exclusively those of cold-blooded animals such as the frog. The selection of cold-blooded animals is guided by the fact that it is possible to isolate the

muscle from the rest of the body and to keep it alive long enough to study its reactions during a considerable time. We shall therefore deal at length with the properties of skeletal muscle, pointing out incidentally in what respects heart muscle and involuntary muscle differ from it.

STRUCTURE OF VOLUNTARY MUSCLE

The voluntary or striated muscles form up to 50 per cent. by weight of the body. Each muscle is embedded in a layer of connective tissue and is made up of an aggregation of muscular fibres 10 to 100μ in diameter, which are united into bundles by means of areolar connective tissue. The individual fibres vary much in length and may be as long as 4 or 5 cm., though usually each fibre is shorter than the bundle of which it forms a part. Some fibres are cylindrical but most of them taper towards one or both ends. At each end of the muscle the fibres are firmly united by their blunt ends to the tendon of the muscles which are attached as a rule to bones. The fine tapering ends of the fibres end in the connective tissue between the fibres. Running in the connective tissue framework of the muscle we find a number of blood vessels, capillaries and nerves.

The living muscle fibre consists of a series of alternate light and dark striæ, arranged at right angles to its long axis and enclosed in a structureless sheath—the sarcolemma (Fig. 34). Lying under the sarcolemma are a number of oval nuclei embedded in a small amount of granular protoplasm. Each band is made up of a number of segments (sarcomeres) side by side, with interstitial substance (sarcoplasm) between them. The sarcomeres are about 2 to 3μ in length, and are connected end to end to form long columns (myofibrils). Each sarcomere is more transparent at the two ends than in the middle, thus giving rise to the appearance of light and dark striæ. In the middle of the light band is a line or row of dots (often appearing double) called Krause's membrane.

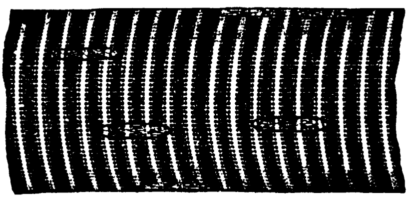

FIG. 34. Muscular Fibre of a Mammal, examined fresh in serum, highly magnified. (SCHAFER.)

The development of this regular cross and longitudinal striation is closely connected with the evolution and specialisation of the muscular function, i e contraction. In many

FIG. 35. Transverse sections of the Pectoral Muscles of *a*, the Falcon, *b*, the Goose, and *c*, the domestic Fowl. It will be noticed that the relative amount of granular or red fibres present varies directly as the bird's power of sustained flight. (After KNOLL.)

cases, as in the developing muscle of the embryo or the adult muscles of many invertebrates, this differentiation affects only part of the cell, so that, while one part presents the ordinary granular appearance, the other half is finely and longitudinally striated, due to the development of special contractile fibrillæ. In the striated muscle of

vertebrates, and still more in the wonderful wing-muscles of insects which can perform three hundred complete contractions in a second, the longitudinal is associated with and subordinated to a transverse striation, due to the regular segmentation of the contractile fibrillæ. The great divergence in the aspect of muscular fibres from different parts of the animal kingdom is largely conditioned by the varying relations, spatial and quantitative. of the sarcoplasm to the fibrils. Thus in the higher vertebrates, two types of voluntary muscular fibre are distinguished, according to the amount of sarcoplasm they contain : one (red fibres) rich in sarcoplasm, more granular in cross section and generally containing a red pigment, myoglobin ; the other (pale fibres) poor in sarcoplasm, clear in cross section and containing no myoglobin. From the fact that the granular fibres are found chiefly in those muscles which have to carry out long continued and powerful contractions, it seems reasonable to regard the interstitial sarcoplasm as the local food-supply of the active myofibrils. The connection between structure and activity of the muscle fibres is well shown by Fig. 35.

In some animals, such as the rabbit, we find muscles consisting almost entirely of one or other of these varieties ; but in most animals the two varieties occur together in one muscle, so that what we have to say about the properties of voluntary muscle really has reference to a muscle containing both red and white fibres. Generally, however, pale muscle predominates in the quick-acting flexor muscles, and red in the tonically acting extensors.

All observers are agreed that the essential contractile element is the chain of sarcomeres forming the muscle fibril. Schafer,* working on the highly differentiated wing-muscle of the wasp, concluded that each myofibril is divided by Krause's membrane, often called the Z-disc (the lines in the middle of each light stripe) into sarcomeres.

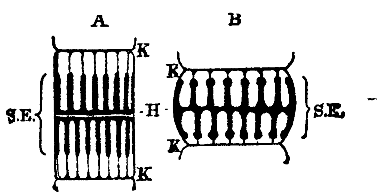

FIG. 36. Diagram of a Sarcomere in a moderately extended condition, A, and in a contracted condition, B; K, K, membranes of Krause; H, line or plane of Hensen; SE, dark stripe. (SCHAFER.)

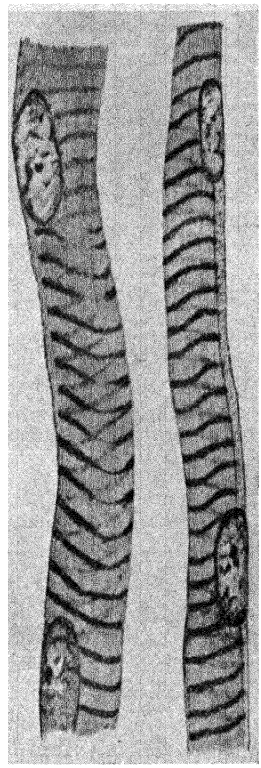

FIG. 37 Two thin muscle fibres of superior oblique muscle of rabbit. The left-hand one shows a double and the right-hand one a single spiral arrangement of striation (TIEGS. *Proc. Roy. Soc.*, 1934, **116B**.)

The Z disc runs right across the fibre from fibril to fibril. Each sarcomere contains a darker substance (Q-disc) divided near the centre into two parts by Hensen's (or Q*h*) disc. At each end of the sarcomere the contents (J) are clear and hyaline. (Fig. 36). Most histologists agree in assigning to the Q disc of the sarcomere a denser structure than to the two ends. Tiegs † considers that the membrane of Krause does not form simple transverse partitions across the fibril, but runs in a spiral form along its whole length as a continuous structure, between the turns of which the sarcomeres are placed (Fig. 37).

When a muscle fibre is examined under the microscope by polarised light, it is seen to be made up of alternate bands of singly and doubly refracting material. The doubly

* SCHAFER. *Proc. Roy. Soc.*, 1891, **49**, 280 ; *Mt. Monatschr. f Anat. u. Phys*, 1891 **8**, 178.
† TIEGS. *Australian J. Exp Biol. Med. Sci.*, 1924, **1**, 11 ; *Proc. Roy. Soc.*, 1934, **116B**, 38.

refracting (*anisotropic*) substance corresponds to the dark (or Q) band, and the Z-disc; the singly refracting (*isotropic*) to the light (or J) band. Actually the light band is also anisotropic, though less so than the dark one.

In the resting fibre of the frog the Q stripe occupies 63 per cent. of the height of the sarcomere and the J stripe 37 per cent. When stretched, the Q stripe extends about twice as much as the J stripe.

On isometric contraction the sarcomere remains unaltered in length and volume, but there is a shrinkage by 18 per cent. of the dark (Q) stripe and an expansion by 28 per cent. of the light (J) stripe.*

Double refraction is generally accepted as due to a substance possessing a regular molecular organisation, *e.g.* crystals show it. There are proofs that the molecules of

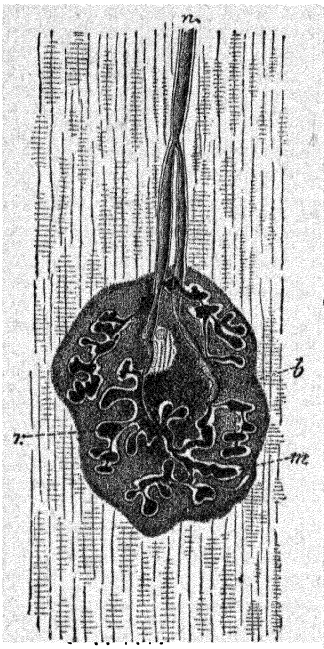

FIG. 38. Motor End Organ of a Lizard, gold preparation. (KÜHNE.)
n, nerve fibre dividing as it approaches the end organ; *r*, ramification of axis cylinder upon *b*, granular bed or sole of the end organ; *m*, clear substance surrounding the ramifications of the axis cylinder.

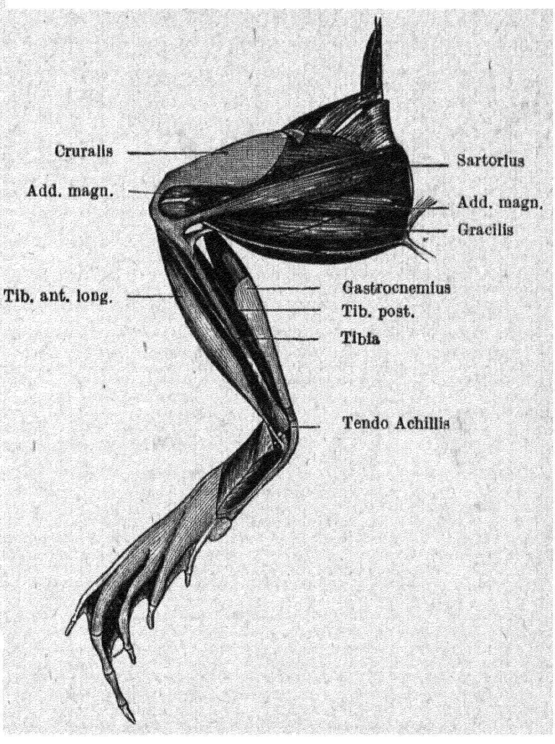

FIG. 39. Muscles of Hinder Extremity of Frog. Ventral Aspect. (After ECKER.)

muscle globulin (myosin) are in the form of long filaments † and that these are arranged parallel to one another in the muscle. At all events, it has been found that while solutions of myosin are flowing, or are being stirred, which would orient the elongated molecules as suggested, the property of double refraction appears. ‡

When a muscle fibre contracts, there is an apparent reversal of the situations of the light and dark stripes, owing to the fact that the interstitial sarcoplasm is squeezed out from between the bulging sarcomeres, and accumulates on each side of the membranes of Krause. The accumulation of sarcoplasm in this situation makes the previously light striæ appear dark, and the dark striæ by contrast

* BUCHTHAL and LINDHARD. "Physiology of Striated Muscle Fibres." 1939. (Copenhagen. Munksgaard).

† STUEBEL. *Pflüger's Arch.*, 1923, **201**, 629.

‡ v. MURALT and EDSALL. *J. Biol. Chem.*, 1930, **89**, 315, 351; *Trans. Faraday Soc.*, 1930, **26**, 837.

lighter than they were before. That there is no true reversal of the striæ is shown by examining the muscle by polarised light, the two substances, isotropic and anisotropic, retaining their relative positions, though the double refraction is reduced. The change in the double refraction has been found by v. Muralt * to follow a curve very similar to that for tension development.

Every skeletal muscle is connected with the central nervous system by so-called motor nerves. The fibres of these are by no means all motor in function, some, often as many as two-fifths of the whole, conveying impressions from the muscle to the centre, some are sympathetic fibres of unknown function, the remainder being motor fibres conveying nerve impulses from the centre to the muscle. These last—the motor nerve fibres, divide into numerous branches when near to the muscle, and each branch terminates in a muscle fibre by means of a special end organ—the motor end plate. The neurilemma of the nerve fibre becomes continuous with the sarcolemma, the medullary sheath ends suddenly, while the axis cylinder ramifies in a mass of undifferentiated protoplasm, containing nuclei and lying in contact with the contractile substance of the muscle immediately under the sarcolemma (Fig. 38). This mass of protoplasm is known as the 'sole plate.' It is not marked in all animals. So far as we can tell at present, the ultimate ramifications of the axis cylinder end freely and do not enter into organic connection with the contractile muscular substance.

Most of our knowledge on the subject of muscle has been derived from the study of the gastrocnemius and sartorius muscles of the frog. The position of these muscles is shown in the accompanying diagram (Fig. 39). The gastrocnemius, with the attached sciatic nerve, is most frequently employed as a *nerve-muscle preparation*. On account of a penniform arrangement of the muscular fibres, the gastrocnemius can be employed only when the contraction of the muscle as a whole is the object of investigation. The effective cross area of the fibres is much greater than the actual cross section of the muscle, so that, while the actual shortening of the gastrocnemius is but small, its strength of contraction is considerable.

The sartorius muscle is of especial value on account of the fact that its fibres are parallel. When a greater mass of approximately parallel fibres is necessary, recourse may be had to a preparation consisting of the gracilis and semimembranosus muscles together. This latter muscle lies dorsally to the gracilis muscle

Other muscles in the frog used for particular purposes are the mylohyoid and the dorsocutaneous muscles. The mylohyoid muscle, which lies on the ventral surface of the tongue, has the advantage that its fibres lie in close contact with a lymph space occupying the centre of the tongue. If any drug be injected into this lymph space it acts with extreme rapidity on the muscle fibres, so that this preparation is useful for the study of the action of different substances on muscle fibres.

Experiments on isolated muscle fibres have often been carried out. They are of value in fundamental observations because of the unequivocal nature of the results obtained.

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RIGOR MORTIS

All muscles after removal from the body, or if left in the body after general death, lose after a time their irritability, and this loss is succeeded

* v. MURALT, *Pflüger's Arch.*, 1932, **230**, 299.

by the phenomenon known as *rigor mortis.* The muscle, which was previously flaccid, shortens, though not very powerfully. Whereas the living muscle is translucent, supple and extensible, it becomes in the process of rigor opaque and much less extensible, the nodulus of elasticity, measured along the fibres, increasing about tenfold.* The rate of onset of rigor is greatly accelerated by treating the muscles with solutions of caffeine, arsenates or chloroform vapour, but is not much affected by temperature.* When rigor has been established, the reaction of the muscle is usually found to have changed from a slightly alkaline (*p*H 7·3) to a distinctly acid one (*p*H 5·8), due to the presence of lactic acid. From this condition of rigor there is no recovery of contractility, though the muscle softens before putrefaction sets in. There can be no doubt that the change in consistence of the muscle, and probably also its shortening in rigor, are due to changes in the muscle proteins. Both conditions can be imitated by heating the muscle. After severe muscular fatigue, as in hunted animals, rigidity may come on almost immediately after death.

EXCITATION OF MUSCLE AND OTHER TISSUES

In order to avoid subsequent repetitions, we shall now consider the excitation of muscle, and at the same time give a general account of the excitability of other tissues, such as nerve, since many phenomena of excitation are common to all tissues.

A muscle may be caused to contract in various ways. Normally it contracts only in response to impulses starting in the central nervous system and transmitted down the nerves. But contraction may be artificially excited in various ways in a muscle *in situ* or freshly removed from the body. If we make a nerve-muscle preparation we find we can cause contraction by various forms of stimuli—mechanical, thermal, or electrical—applied to the muscle or to the nerve (direct and indirect stimulation). Thus the muscle responds with a twitch if we pass an induction shock through it or its nerve, or pinch either with a pair of forceps. Or we may cause contraction by the application of strong glycerol or salt solution to the nerve.

INDEPENDENT EXCITABILITY. These experiments do not prove conclusively that muscle itself is irritable. It might be urged that, when we stimulated the muscle, we really only excited the ramifications of the nerve in the muscle, and that these in their turn excited the muscle to contract. This is indeed usually the case,† because nerve fibres are excited by smaller stimuli than are muscle fibres. But that muscle fibres are capable of independent excitability is shown in the classical experiment by Claude Bernard.‡

The brain of a frog is destroyed, and the sciatic nerves on each side exposed. A ligature is then passed round the right thigh underneath the nerve, and tied tightly so as effectually to close all the blood vessels supplying the limbs, without interfering with the blood supply to the nerve. Two drops of a 1 per cent. solution of curare are then injected into the dorsal lymph sac. After the lapse of a quarter of an hour it is found that the strongest stimuli may be applied to the left sciatic nerve without causing any contraction of the muscles it supplies. On the right side. stimulation of the nerve is as efficacious as before. Both gastrocnemii respond readily to direct stimulation, showing

* SMITH. *J. Physiol*, 1939, **96**, 176.

† ASMUSSEN. *Skand. Arch. f. Physiol*, 1933, **65**, 261

‡ CLAUDE BERNARD. Substances toxiques, p. 344 (Paris, 1857). *Compt. rend.* 1856, **43**, 825.

that the muscles are not affected by the drug. Since both sciatic nerves have been exposed to the influence of the curare, the difference on the two sides cannot be due to any effect on them of the curare. We have also excluded the muscles themselves. So we must conclude that the curare paralyses the muscles by preventing the excitatory process from passing over from the nerve to the muscle, and that muscle can be excited by direct stimulation.

Direct proof of independent excitability of muscle is provided by the fact that individual muscle fibres may be seen under the microscope to contract when stimulated by micro-electrodes at points remote from end-plates.*

ELECTRICAL EXCITATION

The invention of the Leyden jar about the middle of the eighteenth century soon led to the discovery that of all the different stimuli capable of exciting muscular contraction, the electrical is the most satisfactory. This is because the phenomenon of excitation is itself of an electrical nature. It is easy to graduate accurately the intensity and duration of the stimulus, and the stimulus may be applied many times to any point on the muscle or nerve without injury. The four commonest forms of electrical stimuli employed are (1) the make and break of a constant current, such as that from an accumulator, (2) the induction currents of high intensity and short duration obtained from an induction coil, (3) condenser discharges, (4) alternating current.† As the student will have become familiar with these in his practical work, no description of the common forms of apparatus needed for producing these stimuli will be necessary here.‡

RESISTANCE AND IMPEDANCE When a constant current is passed through a tissue, the *resistance*, in accordance with Ohm's law, is,

$$R = E/C,$$

where E is the e.m.f. and C the current intensity. Owing to polarization (p. 165) the current strength rapidly falls off to a low value, because the permeability of the membranes in the tissue to the ions is restricted.

If an alternating current is used less resistance is offered because of the absence of polarization. The resistance to an alternating current is called *impedance*. With an alternating current $I = I_0 \sin \omega t$, where $\omega = 2\pi \times \nu$ (ν = frequency and I_0 = amplitude) the impedance Z includes resistance R and capacitance C. If resistance and capacitance are in parallel, then §

$$Z = \sqrt{\frac{R^2 + \omega^2C^2R^4}{(1 + \omega^2C^2R^2)^2}}$$

GALVANIC STIMULATION. If a weak *constant current* be passed through a muscle or any part of its nerve, at the make of the current the muscle gives a single sharp contraction—a *muscle twitch*, in which, according to the current strength, and other conditions, few or many of its muscle fibres may be involved. During the passage of the current no effect is apparently produced, though on careful observation we may with strong currents see that there is a state of continued contracture limited to the immediate neighbourhood of the cathode, which lasts as long as the current is passing through the muscle, and is not propagated to the rest of the muscle. If the current be now broken, the muscle may remain quiescent, but if the

* GELFAN. *Amer. J. Physiol.*, 1930, **93**, 1.

† KATZ. *J. Physiol.*, 1939, **96**, 202.

‡ *See* D. T. HARRIS. "Experimental Physiology," 1934 (Churchill).
For a precision stimulating device (thyratron tube), *see* SCHMITT and SCHMITT. *Science*, N. S. 1932, **76**, 328.

§ HUBBARD and ROTHSCHILD. *Proc. Roy. Soc.*, 1939, **127B**, 510.

current was above a certain strength, the muscle responds to the break of the current with another single twitch. With a current of moderate strength we may get a contraction both at make and break of the current, *the make stimulus being more effective than the break stimulus.*

Besides this difference in intensity at make and break of the constant current, there is a difference in the point from which excitation starts. *A make contraction starts from the cathode, a break contraction from the anode.* This is called *polar stimulation* and is well shown by the following experiment.

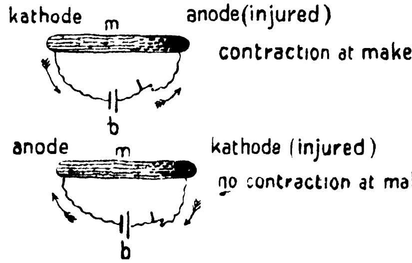

FIG. 40. Diagram to show the Effect of Local Injury on the Excitability of a Muscle. *b*, battery; *m*, muscle. The arrows indicate the direction of the current.

The irritability of a muscle, *i.e.* its power of responding to a stimulus by contracting, is dependent on the life of the muscle. If the muscle be killed at any spot, its irritability at this spot will be destroyed. Hence if we stimulate a muscle at the dead spot, no contraction will ensue. This fact may be used to demonstrate the facts of polar excitation. A muscle with parallel fibres, such as the sartorius, is killed at one end, and a constant current passed, first from the dead to the uninjured end, and then in the reverse direction (Fig. 40). It is found in the former case, when the anode is on the dead part (which is therefore inexcitable), that break of the current is ineffective, and in the latter, when the cathode is on the dead surface, that the make stimulus is ineffective, showing that the part excited corresponds to the cathode at make and to the anode at break.

As will be seen later, the *duration* of the flow of current also affects the result when short periods of flow are concerned. With a current of very short duration no excitation is produced at break. Every induction shock is such a current of very short duration produced in the secondary coil by make or break of the primary circuit, and can be therefore regarded as a make stimulus, and when such a shock is led through a muscle the contraction in each case will start at the cathode, *i.e.* the point at which the induction shock leaves the muscle.

The general laws for stimulation of nerve fibres are similar to those for stimulation of muscle fibres directly.

FARADIC STIMULATION. Using the *induced current* as a stimulus, it is found that the contraction on break of the primary current is much stronger than that on make. It must not be imagined, however, that there is any contradiction between this and the fact that the make of a constant current is a stronger stimulus than the break. It is due to the fact that the break-induced current is, for physical reasons, a stronger one than the make current. Both are currents of very short duration, so short that only the rising phase of each is effective. It has been shown that, when we use currents of such short duration, the fall is ineffective ; so in both cases, whether we make or break the current in the primary circuit, we are dealing with a *make* stimulus in the muscle.

In using either of these modes of stimulation we find that there is a certain intensity which the stimulating current must possess in order that any effect shall be produced. Any strength of stimulus below this is known as a *subliminal* stimulus. A *liminal* stimulus (sometimes known as *minimal* or *threshold* stimulus) is the weakest stimulus that will produce any result, *i.e.* in muscle a contraction. With galvanic excitation, this

is also called the *rheobasic strength*. A *maximal* stimulus is one that produces the strongest contraction of which a muscle is capable as a result of a single stimulus. A *submaximal* stimulus is any strength of stimulus between these two extremes.

TIME FACTOR IN EXCITABILITY. Examination of the properties of different living tissues demonstrates that their excitabilities vary widely. Some tissues require on galvanic stimulation a much stronger current than others, or, on faradic stimulation, a closer coupling of the secondary to the primary coil. Again, some respond to galvanic but not to faradic excitation, or *vice versa*. It is clear that comparison of excitabilities in different tissues is unsatisfactory, if at all possible, on such indications as these. In view of the fact that there is no fundamental qualitative difference between galvanic and faradic currents, such a statement as "excitable to faradic, inexcitable to galvanic electricity," so often met with, is meaningless.

Dealing with galvanic stimulation as more readily analysable than

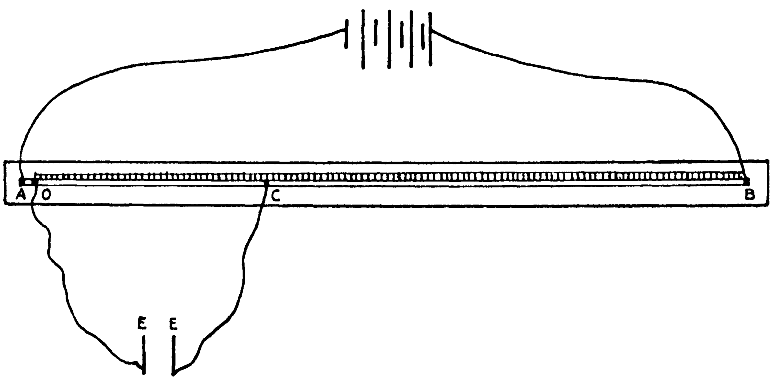

FIG 41. Simple Liquid Potentiometer.

faradic, it is found that the following must be taken into account in studying excitability :—

(*a*) *The current density* or strength of current (*c*) or else the *voltage* (V) — to which (*c*) is proportional—required to stimulate.

(*b*) *The duration of flow* of the current. It has been found that a current that will just excite when of a particular duration, fails to do so when its duration is reduced below a certain level ; also, for a given current, further continuation of the current, beyond a particular duration, differing for each tissue, produces no further effect.

An elementary illustration, given by Lapicque, will serve to show the importance of the time factor in excitation. A liquid potentiometer is used consisting of a long narrow trough filled with zinc sulphate solution. Into it dip two plates of amalgamated zinc A and B (Fig. 41), placed about one metre apart and connected with the source of current ; two other similar plates also dip into the gutter ; O is stationary and near to A, while C can be dipped in and out of the solution, thus making and breaking contact. The electrodes EE are placed in contact with a strip of plain muscle (frog's stomach). The strength of current flowing through the tissue is proportional to the distance of C from O. If C be plunged into the groove at intervals of a few seconds, further and further from O at each immersion, a point, the *threshold* will be reached, at which each prolonged immersion is followed by contraction of the plain muscle. The distance OC at which this occurs (say

10 cm.) is noted. Now C is withdrawn and dipped as quickly as possible in and out of the groove at the same point, giving stimuli lasting only about one-tenth of a second; no contraction will result. If the distance OC is made greater, however (say 70 cm.), so as to increase the potential tapped off, even this brief duration will stimulate.

(*c*) The *rate of change* of the current strength. In (*a*) and (*b*) it has been assumed that the current was established and stopped instantaneously ("rectilinear waves") as in Fig. 42 (A), whereas under certain circumstances a current might fluctuate by a slope, *e.g.* as shown in Fig. 42 (B), and these fluctuations would need to be taken into account. If, instead of plain muscle, the nerve of a frog's sciatic-gastrocnemius preparation be placed on the electrodes EE, and the cursor C of Fig. 41 be *quickly* moved along the groove, say, from O to 20 cm., so as to cause a rapidly increasing current to flow through the nerve, the latter will be stimulated as shown by contraction of the muscle. Now return the cursor to O and move it away *very slowly*; it will be found that although it be moved far beyond the previous distance (say to 50 cm.), no contraction will follow—the slow slope of current-increase is ineffective, whereas the steep gradient stimulated the nerve.

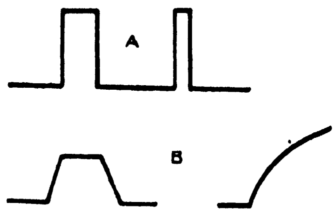

FIG. 42.

Du Bois Reymond expressed this fact by stating that excitation depends on the rate of variation of current density from moment to moment; the more rapid the change the greater the excitatory effect.

This statement is now known to be only partially true. We should now regard it as more correct to say that the current excites during the first moments of its passage, whether variable or constant, but that a certain duration of flow is essential.

To demonstrate the importance of duration in the stimulation of such highly excitable tissues as nerve or striated muscle we require much more refined apparatus than that described above for use with the relatively inexcitable plain muscle. If we can get currents of sufficiently short duration, we can demonstrate that with *any* tissue a liminal current which will excite at a longer duration fails to do so when it is abbreviated beyond a certain limiting value. This limit for the frog's stomach is of the order of tenths of a second, while for the sciatic nerve it is of the order of thousandths of a second.

These simple considerations serve to illustrate the importance of the time factor in excitation, and to show that "slow" and "rapid" are purely relative terms when different tissues are under consideration.

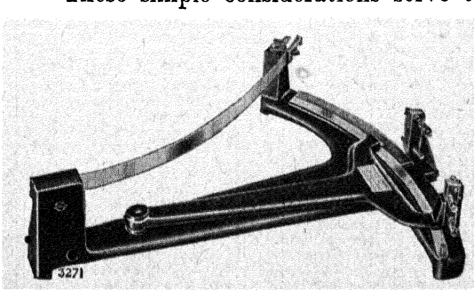

FIG. 43. Keith Lucas's Spring Rheotome.

Currents of sufficiently short duration can be obtained by the use of a mechanical device or rheotome, such as Lucas's pendulum or spring (Fig. 43), by which two keys are opened at known intervals. The first key is a short circuiting key, the opening of which starts the current, and the second one is in series, and its opening stops the current.

Another method is by the use of condenser discharges. The time taken for a condenser to discharge can be calculated if its capacity and the resistance of the circuit are known, the time being proportional to the product RC. The current strength is, of course, not constant during the discharge, but it is much easier to obtain currents of very short duration by the adjustment of condenser capacities than by any of the rheotomes for use with constant currents, and the drawbacks attendant on the use of discharges of rapidly declining strength have been overcome, since it has been shown that a discharge from a condenser charged to a given voltage is equivalent to a rectilinear constant current of duration = 0·37 RC seconds, at the same voltage (R = resistance of circuit in ohms ; C = capacity of condenser in farads).

When the smallest voltages which will excite a tissue are plotted against the durations of the current, controlled in either of these ways, a curve of the rectangular hyperbola type (Fig. 44) is obtained, one of the asymptotes

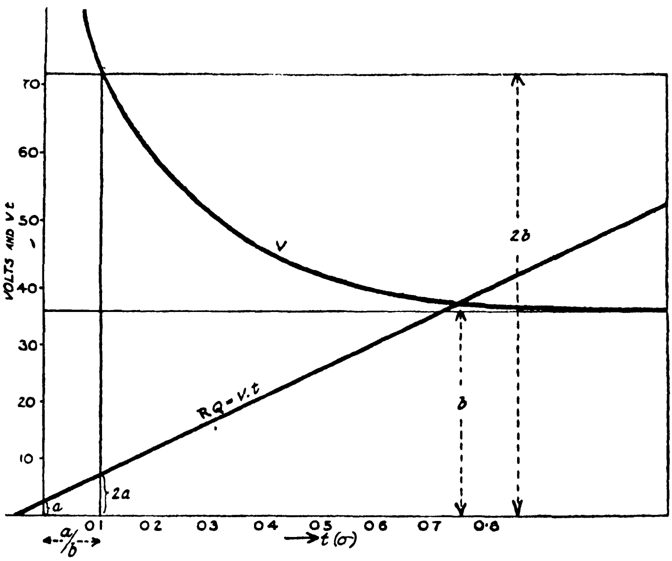

FIG. 44. To illustrate Weiss's Laws and their Relation to Chronaxie. V = curve of voltages. RQ = curve of quantities. $a = 3\ 5$. $b = 36$ volts. $\frac{a}{b} = c\ 0{\cdot}1\sigma$ = chronaxie. Abscissæ = time in $0{\cdot}1\sigma$. Ordinates figured = volts. Ordinates for RQ = Vt are on same scale. (Modified from BOURGUIGNON. *La Chronaxie.*)

of which is a line at the level of the threshold voltage (b) for currents of infinite duration. Thus in Fig. 44, a voltage lower than b (= 36 v.) will not stimulate that particular tissue under the given conditions of experiment ; a current of 40 v. will stimulate if it lasts for not less than $0{\cdot}55\sigma$, one of 50 v. if it lasts for at least $0{\cdot}35\sigma$, &c.

For a different tissue, and with other conditions of experiment, quite different voltages and times would probably be obtained, but the form of the curve would always be the same, though the scales would differ. Such curves are called *strength-duration curves.* An important condition in the preparation of these is that all the conditions of experiment, except strength and duration of the exciting current, must remain the same throughout any given curve.

If the quantities of electricity (proportional to Vt) are plotted against times, a straight line is obtained.

These relations for constant currents are expressed by Weiss's laws :—

(1) For intensity,

$$i = \frac{V}{R} = \frac{a}{t} + b.$$

(2) For quantity,

$$Q = it = a + bt,$$

where a and b are two constants.

a is the quantity of electricity where the graph cuts the ordinate.

b is the threshold strength at infinitely long duration. Almost identical relations are obtained by the use of condensers (Hoorweg's laws).

In a strength-duration curve, current strengths are plotted against the *excitation-times*, *i.e.* against the shortest times in which each strength can excite. Evidently, if other things were equal, the more excitable a tissue the shorter the time needed to rouse it to respond.

Lapicque calls the liminal value for indefinite duration (galvanic threshold) the *rheobase*, and estimates the excitability of a tissue by what he has called the *chronaxie*. This is one specially defined excitation-time.

The chronaxie may be defined as the shortest duration of a current of twice the rheobasic strength which will stimulate. Thus in Fig. 44, when the voltage is raised to $2b$, the shortest duration which will excite is $0{\cdot}1\sigma$, and this duration is the chronaxie. A less excitable tissue has a longer, and a more excitable one a shorter chronaxie.

In practice, in order to determine the chronaxie, all that is necessary is to find the galvanic threshold or rheobase, using an ordinary key, then double the voltage so employed, and finally, using either condensers or a suitable rheotome, find the shortest duration of this which will excite.

Provided the conditions of experiment are constant, the chronaxie of a tissue is a definite measure of its excitability, since in this time a current of twice the rheobasic strength is able to produce those physico-chemical alterations which determine excitation, and the more excitable the tissue is, the more quickly will that current produce those changes. It can be shown that what happens in an excitable tissue in, say 3/10,000 of a second, may require, say, eight seconds for a very inexcitable one.

Whether it is feasible to obtain constant conditions from one experiment to another, or even throughout a single experiment, is, however, a matter of opinion, which has been contested with some heat. Variation in the size or nature of the stimulating electrodes is especially liable to produce widely different results.*

The following table shows some approximate chronaxies for excitable tissues of very varying types :—

Tissue.	Chronaxie (σ).	Approximate Duration of Contraction of Muscles.
		Seconds.
Frog's sciatic and gastrocnemius (esculenta)	0·3 σ	0·1
,, ,, ,, (temporaria)	0·7 σ	0·1
Toad's sciatic gastrocnemius	1·3 σ	0·16
Frog's ventricle	3·5 σ	1·0–2·0
Tortoise heart (testudo græca) . . .	8·2 σ	2–3
Claw muscle of crab	30 σ	5
Frog stomach	30–100 σ	15–20
Pigment cells, skin of frog	1000–15,000	—

* For a criticism of the experimental facts regarding chronaxie, see Rushton. *Journ. Physiol.*, 1932, **74**, 424 ; **75**, 445.

It is evident from consideration of the figures for different chronaxies that there is a relation between chronaxie and speed of action of tissue. The more excitable tissue has, as a rule, a shorter chronaxie, shorter period of action, shorter refractory period, and a quicker rate of propagation than a less excitable one.

It is because of its very short duration ($0{\cdot}1\sigma$) that the induced current is unable to effect the excitation of sluggish tissues such as some forms of plain muscle. When the duration is made extremely short, even very high voltages fail to excite any tissues. Hence high frequency alternating currents of enormous voltage can be passed through the body without any physiological effect other than that due to the heating effects, as in the practice of diathermy.

According to Lapicque, the same chronaxie is found for a muscle stimulated directly or *via* its nerve. The classical view, which has been upheld by Rushton, was that the muscle is much less excitable than its motor nerve, and that when the "muscle" is

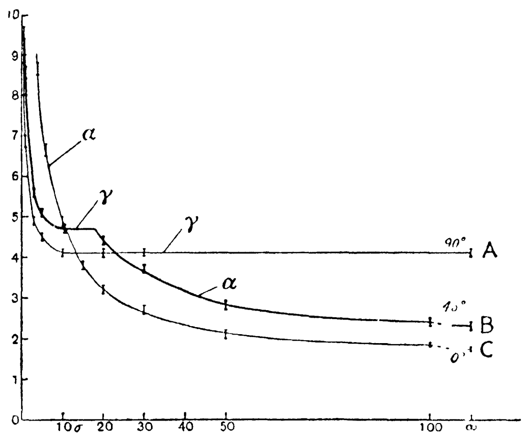

FIG. 45.—Strength-duration curves from sterno-cutaneous muscle of frog stimulated at 90°, 45° and 0° to direction of muscle fibres. (RUSHTON, *J. Physiol.*, 1930, **70**, 334.)

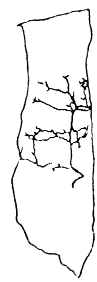

FIG. 46.—Sterno-cutaneous muscle of frog showing direction of nerve fibres (RUSHTON, *J. Physiol.*, 1932, **75**, 174.)

stimulated it is really the nerves within it which are excited and these then stimulate the muscle fibres. In support of this opinion he adduces the α and γ curves of the nerve-muscle complex which were discovered by Keith Lucas. This observer found that the strength-duration curve of a muscle (with its contained nerve branches) was composite, as shown for curve B (Fig. 45), and supposed this to be due to the presence of a more excitable (or γ) tissue, which was probably nerve, and of a less excitable one (α), probably muscle. Lapicque, however, obtained a uniform curve resembling C, of properties akin to Lucas's α-curve. Rushton showed that in a parallel-fibred muscle either α- or γ-, or composite curves could be obtained, according to the angle at which the stimulating current passed relatively to the direction of the muscle and nerve fibres. This is because muscle and nerve fibres are most excitable when stimulated by currents parallel to their fibres, and least excitable to currents running at 90° to their fibre direction (more precisely the rheobase varies inversely as the cosine of the angle between current direction and fibre direction). Whether a given current tends to excite the muscle fibres or the intra-muscular nerve fibres will therefore depend not only on its strength and duration, but also on its relation to the direction of the muscle and nerve fibres. In many muscles a majority of the small nerves run roughly at right angles to the muscle fibres (Fig. 46). Hence if, as in Fig. 45 C, we stimulate with a current parallel to the muscle fibres we get the muscle or α curve, if at 90° the γ or nerve-excitation curve (Fig. 45 A), and if at 45° a composite curve (B).

If the intra-muscular nerves are dissected away, or if curare is given, the curve becomes a smooth α-curve, characteristic of muscle, under all circumstances.

Lapicque's explanation is entirely different. He believes that unless the nerve and muscle have *approximately* the same chronaxie the excitatory state will not be transmitted (law of isochronism): this failure occurs when the chronaxie of one tissue is more than twice that of the other. Curari, he claims, increases the chronaxie of muscle but not that of nerve; strychnine on the other hand reduces that of nerve but leaves the muscle unaltered: in both cases conductivity from nerve to muscle fails. He claims that the conditions of Rushton's experiments are not such as would be expected to give true chronaxies.

Latent Addition. Closely associated with the excitability of the tissues are the phenomena of latent addition of excitation, or '*summation of stimuli*' and of '*refractory period*.' If two *subliminal* stimuli are sent in within a sufficiently short interval of time, their effect is summated so that two stimuli, each of which would be ineffective, may together produce an excitation. For summation of two stimuli to take place, the second stimulus must occur at a time before the condition excited by the first stimulus has died away. The maximum time interval at which summation can take place will therefore vary from tissue to tissue, and will bear a relation to the chronaxie. This is evident if we compare the maximum summation intervals for different tissues with their chronaxies. Thus for frog's nerve, with a chronaxie of 0 3 σ, the summation interval is 0·5 σ, while for frog's heart, with a chronaxie of about 3 5 σ, the maximal summation interval is 8 0 σ.

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THE MECHANICAL CHANGES THAT A MUSCLE UNDERGOES WHEN IT CONTRACTS

If a skeletal muscle, such as the gastrocnemius, be stimulated by a single induction shock either directly or by the intermediation of its nerve, it responds by a single short, sharp contraction, followed immediately by a relaxation. In volume the muscle undergoes a very slight, and for most purposes negligible, shrinkage,* but each muscle fibre, and the whole muscle, become shorter and thicker. At the same time, if a weight be tied on to the tendon of the muscle, the muscle during contraction may raise the weight and thus perform mechanical work. To determine the time relations of the simple muscle contraction or twitch, and to study its conditions, it is necessary to employ the graphic method.

ISOTONIC CONTRACTION. In order to record the shortening of the frog's gastrocnemius, the muscle is excised together with a portion of the femur to which it is attached, and often with the whole length of the sciatic nerve. The femur is clamped firmly, and the tendo Achillis attached by a thread to a light lever, free to move round an axis at one end. The point of this lever is armed with a writing point, which just touches the blackened surface of a piece of glazed paper stretched round a cylinder (drum) which can be made to rotate at a constant speed. If the drum is moving, the point draws a horizontal white line or abscissa on the smoked paper.

* Meyerhof and Möhle. *Bioch. Ztschr.*, 1933, **260**, 454, 469; **261**, 252.

If a single induction shock be sent through the muscle (Fig. 47), or nerve, the lever is jerked up, falling again almost directly, and a curve is drawn like that shown in Fig. 48.

In all such graphic records we should have also—

(1) *A time record.* This is furnished by means of a small electro-magnet, armed with a pointed lever writing on the smoked surface. This electro-magnet (time marker or signal) is made to vibrate 100 times a second (more or less as may be required) by

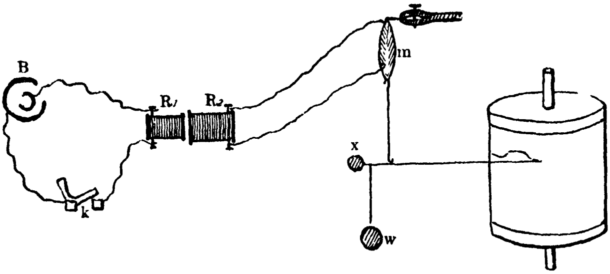

FIG. 47. Arrangement of Apparatus for recording simple Muscle Twitch.

putting it in a circuit which is made and broken 100 times a second by means of a tuning fork vibrating at that rate.

(2) *A record of the exact point at which the nerve or muscle is stimulated.* This may be obtained in two ways:

(*a*) A trigger key is so arranged that a projecting arm of the drum strikes it, and breaks the primary current of an induction coil at a pre-determined point as the drum revolves.

(*b*) By means of an electro-magnetic signal, interposed in the circuit, and arranged to write on the revolving drum.

In Fig. 48 the upper line is the curve drawn by the lever of the muscle as it

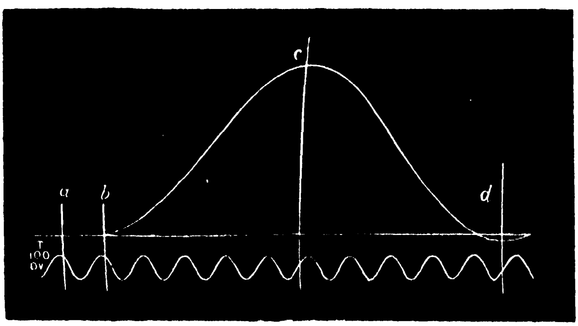

FIG. 48. Curve of simple Muscle Twitch. *a*, point of stimulation; *ab*, latent period; *bc*, period of contraction; *cd*, period of relaxation. Time = $\frac{1}{100}$ secs. (After STIRLING.)

contracts; the small upright line *a* shows the point at which the muscle was stimulated; and the lower line is the tracing of the chronograph, every vibration representing $\frac{1}{100}$ of a second.

The graphic record may, in consequence of instrumental inertia, be a very inaccurate reproduction of the true events occurring in the muscle itself. When the muscle begins to contract, it imparts a very rapid movement to the lever which therefore tends to overshoot the mark and deform the curve. This source of error may be reduced by making the lever as light as possible, and hanging the extending weight in close proximity to the axle of the lever, as shown in Fig. 48. Since the energy of a moving mass is proportional to the square of the velocity ($= \frac{1}{2}mv^2$) and the tension due to the weight as well as the velocity imparted to the weight on contraction is

directly proportional to the distance of the weight from the axis, it follows that it is better to load the muscle with 40 grammes 1 millimetre from the axis than with 1 gramme 40 millimetres from the axis. though the tension put on the muscle will be the same in both cases.

In the first case the energy of the moving mass will be proportional to $\frac{40 \times (1)^2}{2} = 20$, and in the second to $\frac{1 \times (40)^2}{2} = 800$, and it is this energy which determines the overshooting of the lever and the deformation of the curve.

In the method just described, the lever follows the muscle in its movement, the tension on the muscle remains the same throughout, and the contraction is therefore known as an *isotonic* one. A simple isotonic contraction or twitch, such as that in Fig. 48, produced by single stimulus, consists of three main phases :

(1) A phase during which no apparent change takes place in the muscle. This is called the *latent period*.

(2) A phase of shortening or *contraction*.

(3) A phase of *relaxation* or return to the original length.

The small curve seen after the main curve is due to elastic vibrations of the lever. The latent period for the frog gastrocnemius as usually recorded occupies about 10 msec., the phase of shortening 40 msec. and the relaxation 50 msec.

Thus a single muscle twitch in a frog is completed in about $\frac{1}{10}$ second. It must be remembered, however, that this figure is only approximate, and varies with the temperature of the muscle and its condition, being much longer in a fatigued muscle. It depends also on the animal from which the muscle is taken, and on the particular muscle. In mammals it is not necessarily shorter than in the frog. Thus in the cat, the period of actual *contraction* is, soleus 100 msec., gastrocnemius 40 msec., internal rectus of eye 8 msec. In the tortoise a single twitch may last from half a second to five seconds, according to temperature. In the wing muscle of a wasp the duration of a single contraction may be only 5 msec

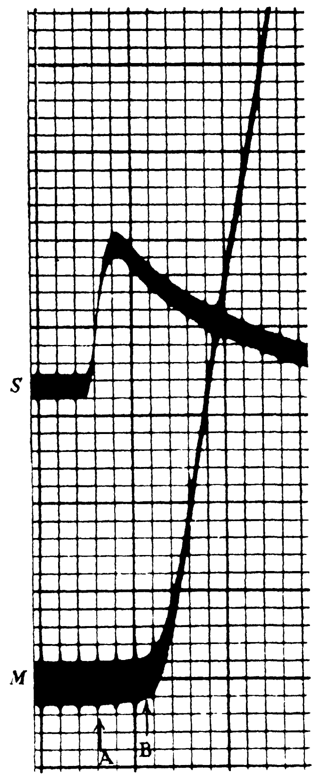

FIG. 49. Photographic Record of Contraction of Frog Muscle. *S* = signal of stimulation. *M* = mechanical change in muscle. Abscissa divisions = 1 msec. First phase of slight shortening begins at A, second phase, rapid shortening at B. Time to A is less than ½ msec. Time to B, about 3 msec. (Roos.)

It is almost impossible to avoid some deformation of the curve due to defects of the recording instruments used. Thus the period during which no mechanical changes are taking place in the muscle must always be shorter than is apparent from a curve obtained by the foregoing method. The viscosity and extensibility of the muscle must prolong this apparent latent period, since the first effect of contraction of any part of the muscle will be to stretch the adjacent part, and only later to move the tendon to which the lever is attached. If we have a weight suspended by a piece of elastic, it will not follow a pull exactly but will lag behind, the first part of the pull being occupied with stretching the indiarubber, and only when this is stretched to a certain degree will the weight begin to rise. The same retardation of the pull would be observed if, instead of indiarubber, we used a piece of living muscle.

It is possible to obviate this instrumental inertia by employing photographic

methods for the record. Such experiments were carried out long ago by Burdon-Sanderson, and show that the true latent period is extremely short.

According to Einthoven* and also Roos† the interval between the moment of stimulation and the onset of mechanical changes is even shorter, less than 0·4 msec, if inertia is eliminated as much as possible (Fig. 49); probably action current and shortening begin together (Fig. 50), or at all events the commencement of the action potential does not precede the commencement of the shortening by more than a short interval. The first part of the shortening curve shows only slight change, and it is a matter for debate whether this phase should not be considered as part of the latent period, thereby lengthening it by about 3 msec. (Fig. 50).

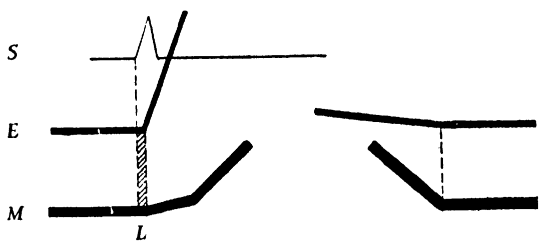

FIG 50. Diagram of time relations of stimulus, electrogram and mechanogram of skeletal muscle S = stimulus, E = electrogram, M = mechanogram, L = latent period (0 1-0 4 msec) Beginning and end of the curves are shown (Roos)

The relaxation of muscle is helped by a moderate load, and in a normal condition is complete. It is not active—that is to say, is not due to a contraction in the transverse direction—but is a passive effect of extension. This may be shown by allowing a muscle to contract while floating on mercury when the subsequent lengthening on relaxation is very incomplete.

ISOMETRIC CONTRACTION. It is of importance to be able also to record the development of the energy (*i e.* the tension) of the active

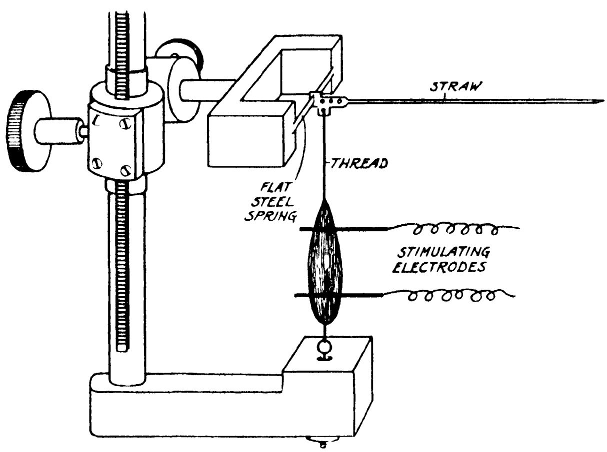

FIG 51 Isometric lever on torsion principle The steel torsion-spring is fixed firmly between the square jaws, and a lever fastened to it indicates the extent of the twist given when the muscle, attached to the lever as near as possible to its attachment to the wire, is caused to contract. (From WINTON and BAYLISS's "Human Physiology")

muscle apart from any changes in its length. For this purpose the muscle is allowed to contract against a strong spring, the movements of which are magnified. Thus the shortening of the muscle is almost entirely prevented,

* EINTHOVEN. Harvey Lectures, 1924-1925 p. 111.
† ROOS. *J. Physiol.* 1932 **74**, 17.

but the increase in its tension causes a minute but proportionate movement of the spring, which is suitably recorded. Since in this case the length of the muscle remains approximately constant throughout, it is known as the *isometric* method. The great magnification necessary in this method necessitates the use of instruments having low inertias and very high natural vibration frequencies (about 1000-2000 per second), as otherwise the record is deformed by the vibrations of the spring. The best arrangements are on the torsion-wire principle, one type of which is shown in Fig. 51.

The *isometric twitch* has a form roughly similar to that of the isotonic contraction, but differing from it in detail. The rise of tension commences very abruptly about 1 msec. after the beginning of the electrical response (mammal), the curve showing at first an upward concavity, soon followed by an upward convexity. After reaching the summit, there is an upwardly concave passive relaxation (Fig. 52). The duration of the contraction process is given by the interval between the onset of electrical response and the summit of tension production.

Very considerable tensions are produced in isometric contractions; several hundred grammes with a small frog's gastrocnemius. The duration

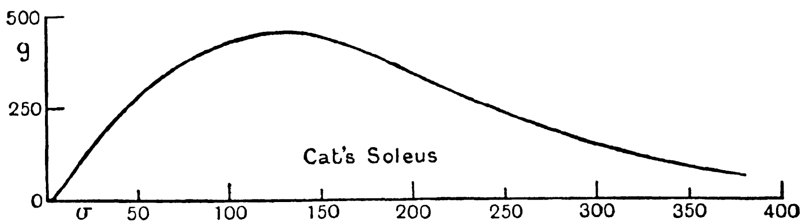

FIG. 52. Isometric Contraction of Cat's Soleus Muscle. Abscissæ = time in σ. Ordinates = grammes tension (COOPER and ECCLES, *Journ. Physiol.*)

of the contraction process is definitely *longer* in an isometric twitch than when the muscle is allowed to shorten (isotonic contraction); the duration is also increased by increasing the "initial" tension on the muscle, *i.e.* the stretch given to the muscle before its stimulation.

Isometric contractions have been recorded in single fibres.*

EFFECT OF TEMPERATURE. When a muscle is moderately warmed, the chemical processes involved in the phase of shortening as well as those responsible for relaxation are hurried up, while the viscosity is diminished. The latent period therefore becomes shorter and the muscle curve steeper and shorter.

It is very often observed that in an isotonic twitch the height of the curve of contraction of the warmed muscle is greater than that obtained at ordinary temperatures. This apparent increase in height is really instrumental in origin, the quicker moving muscle jerking the lever beyond the real extent of the contraction.

When isometric contractions are recorded, it is found, at any rate in the frog, (1) that the tension developed in a single twitch diminishes slightly as the temperature is raised from 0° C. to 25° C.; (2) that the tension developed in a tetanus lasting a sufficient time to attain its maximum is increased with rise of temperature, but not to a very marked extent; (3) that the durations of the latent period and contractile process are greatly reduced, and the duration of relaxation somewhat reduced by rise of temperature.

* BROWN and SICHEL. *J. Cell. and Comp. Phys.*, 1936, **8**, 315.

If a muscle be heated gradually (without stimulation) up to about 45° C., it begins to contract slowly at about 34° C., and this contraction reaches its maximum at 45° C., at which point the muscle has entered into pronounced *rigor mortis*. This is often called heat rigor.

Cold has the reverse effect. All the processes of muscular activity are slowed, so that the latent period and the contraction period are prolonged. Slight cooling also lowers the *threshold of excitability* of muscle, so that any form of stimulus is more effective at 5° C. than at 25° C. If a muscle be cooled for a short time to zero or a little below, it loses its irritability, which returns if the muscle be gradually warmed again. Prolonged exposure to severe cold irrevocably destroys its irritability, and warming the muscle will now simply bring about *rigor mortis*.

PROPAGATION OF CONTRACTION. The whole muscle does not, as a rule, contract simultaneously. When excited from its nerve the contraction begins at the end plates and spreads in both directions through the muscle. The rate of propagation of the contraction wave can be measured only by employing a curarised muscle, so as to avoid the wide spreading of the excitatory change by means of the intra-muscular nerve twigs. For this purpose a curarised sartorius muscle is stimulated at one end, and the electrical variation in the muscle recorded by two pairs of electrodes, one (*a*) placed near the exciting electrodes and the second (*b*) at the other end of the muscle. The difference between the times of commencement of the electrical variations at the two places gives the time taken by the contraction wave in travelling from *a* to *b*.

By measurements carried out in this way it is found that the rate of propagation of the contraction in frog's muscle is 3 to 4 metres per second; in the muscle of warm-blooded animals it may amount to 6 to 12 metres. The *length of the wave* is obtained by multiplying the rate of transmission by the duration of the wave at any one point. It varies in frog's muscle between 150 and 360 millimetres. Thus the muscle fibres are much too short to accommodate the whole length of the wave, and the contraction of the whole muscle must be made up of the summated effects of the contraction wave as it passes from point to point.

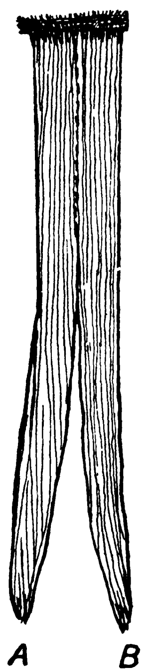

FIG. 53.

THE EFFECT OF STRENGTH OF STIMULUS. THE 'ALL-OR-NONE' RULE. If a series of single break induction shocks be applied to a muscle at intervals of not less than five seconds, it will be found that when these are below a certain strength no effect at all is produced. The shocks are said to be *subliminal*. On gradually increasing the strength a point will be reached at which a small contraction will be observed (*liminal* or *threshold* stimulus). Further steady increase in the strength of stimulus will now result in a gradual increase in the power of the contractions (*submaximal stimuli*) until finally a maximum response is reached (*maximal stimulus*); further increase in the strength of stimuli (*supra-maximal stimuli*) cause no further increase in contraction, although the current actually used may be increased a hundred times. It might seem that this limited gradation of the muscular response according to strength of stimulus was due to a similar gradation in the response of each of the muscle fibres of which the muscle is composed. The fact is, however, that when a minimal or submaximal response is obtained, not all the fibres making up the muscle are contracting. A minimal contraction is, in fact, a contraction in which *a few* fibres of the whole muscle are stimulated. A maximal contraction is one in which *all* the fibres are stimulated. So far as concerns each individual muscle fibre

every contraction is probably a maximal contraction, *i.e.* the rule of 'all-or-none' which was first enunciated for heart muscle is probably true for skeletal muscle. The difference between skeletal and heart muscle lies in the fact that in the former the excitatory process does not spread from one fibre to its neighbours. If, for instance, we take a curarised sartorius and split its lower end as in Fig. 53, the stimulus applied to A causes a contraction only of the left side of the muscle, while a stimulus applied to B is in the same way limited to the right side. If a piece of ventricular or auricular muscle of the frog or tortoise were treated in the same way, a stimulus applied at A would cause a contraction which would extend to B.

The 'all-or-none' law does not mean that it is impossible to vary *by any means* the extent of the mechanical response of a fibre to stimulation, but only that such variations cannot be brought about by altering the strength of the stimulus.

The 'all-or-none' character of muscular contraction was indicated by Gotch and Keith Lucas,* but the clearest demonstration of it is that given by the experiments

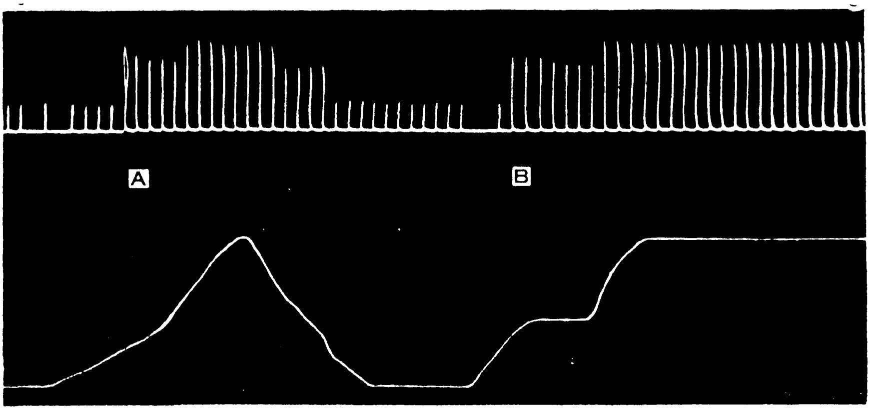

FIG. 54. Record of Contraction of Sartorius of Frog stimulated by break shocks from a 'pore' electrode. The record was made by photographing the excursion of a droplet of mercury on the surface of the muscle. Upper tracing = extent of successive contractions; lower tracing = variation in intensity of stimulus. (After PRATT and EISENBERGER.)

of Pratt and Eisenberger.† These investigators were able, by the use of micro-electrodes, to apply stimuli to individual fibres, while observing these directly under the microscope, and also to take photomicrographic records of the extent of the contractions which resulted. One of these records is shown in Fig. 54. It will be noticed that, as the strength of the stimulus is steadily increased, the response increases in a series of steps, each of which was seen to correspond to the occurrence of contraction in a fresh fibre or fibres which had been unaffected by the weaker stimuli: each fibre which enters into contraction does so with maximal strength. The grading of muscular power is thus achieved by variation in the number of fibres or parts of fibres brought into play. Further investigation of the phenomenon by other workers suggests that this simple view may need to be qualified, since when very fine electrodes are used, the response in their immediate neighbourhood is graded.‡ This is apparently due to the fact that weak and very localised stimulation of a single fibre causes a localised contraction which does not propagate and occupy the whole fibre.§

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* LUCAS. *J. Physiol.*, 1909, **38**, 113.
† PRATT and EISENBERGER. *Amer. Journ. Physiol.*, 1919, **49**, 1.
‡ v. GELFAN and GERARD. *Amer. J. Physiol.*, 1930, **95**, 412.
§ PRATT. *Amer. J. Physiol.*, 1930, **93**, 680.

THE REPETITION OF STIMULI

SUMMATION OF CONTRACTIONS AND TETANUS. The response of a single muscle fibre to a single shock, whether measured by the isotonic or the isometric method, *i.e.* as shortening or as tension, is independent of the strength of stimulus and varies only with the length of the fibre. If, however, a second shock is sent in during the rise of the excitatory condition a further evolution of energy is possible, and the effect is still further increased by putting a series of stimuli into the muscle or its attached nerve before the development of the contractile stress due to the previous stimulus has reached its maximum. If two shocks at intervals of, say, one fiftieth of a second be sent into a muscle, the response will be greater than that produced by one shock (Fig. 55). The effects of the two stimuli are summated, but the resulting contraction is usually less than twice the size of the simple twitch, though it may be more.* (This phenomenon of *summation of contractions* must be clearly distinguished from that of summation of stimuli referred to previously.) If a series of shocks be sent in, the excitatory condition is maintained, so that instead of a simple muscle twitch, rising to a maximum and then falling, the muscle lever rises to a given point (Figs. 56 and 57), which in the muscle contracting isometrically may be four times that due to a single stimulus and then remains at this height during the continuance of the repeated excitations. The state of apparently continuous contraction evoked in a muscle by repeated shocks is called a *tetanus* (Fig. 56, D). If the stimuli are not rapid enough to give complete fusion, a partially maintained con-

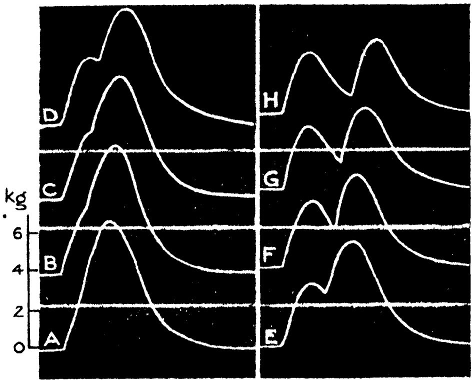

FIG. 55 Isometric Responses of Median Head of Gastrocnemius of Cat, *in situ*, to two maximal stimuli to peroneal nerve.
The intervals between the two shocks were lengthened in order from A (shortest interval) to H (longest interval). The times between the first and second shocks were, in msec : A, 24 ; B, 32 ; C, 40 ; D, 48 ; E, 57 ; F, 69 ; G, 77 ; H, 88. Time intervals marked are 20 msec. (After COOPER and ECCLES, *Journ. Physiol.*, 1930, **69**, 377.)

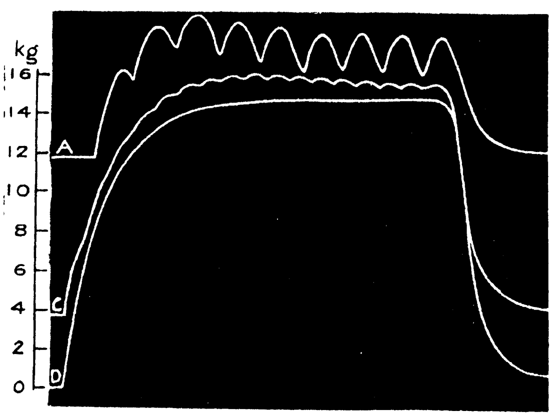

FIG. 56. Isometric Responses of Median Head of Cat's Gastrocnemius to rapidly interrupted Repetitive Stimulation of Popliteal Nerve. A, at 19 ; C at 35 ; D at 115, shocks per second. (COOPER and ECCLES.)

* COOPER and ECCLES. *Journ. Physiol.*, 1930, **69**, 377.

traction, called incomplete tetanus, or *clonus*, results (Fig. 56, A, C.). The rate necessary to give tetanus is higher the shorter the contraction time of the muscle, and therefore varies from muscle to muscle. If the muscle be allowed to contract isotonically, the continued contraction produced by a series of stimuli may with a heavy load be three or four times as great as that produced by a single stimulus.

When, as under the conditions of ordinary life, the muscle contracts in response to impulses transmitted to it by its motor nerve, the conditions are not quite the same as when the muscle is stimulated directly. Each motor nerve fibre supplies many muscle fibres, often about 150.* The unit of response is therefore a nerve fibre with all the muscle fibres which it supplies ; this is called a *motor unit*.

When a motor nerve is stimulated electrically with a maximal shock we may suppose that all the motor units are thrown into action If a second shock follows quickly on the first, and both are maximal, we get summation, as explained above. The same phenomenon is seen when the stimuli are submaximal.

This type of summation may be called *wave-summation*, because it is due to the fusion of successive waves of contraction in the same fibres. This summation is not algebraical, *i.e.* the second contraction does not exactly double the shortening brought about by the first, nor does it double the duration ; the effect of the second stimulus is greater the earlier it falls during the first response.

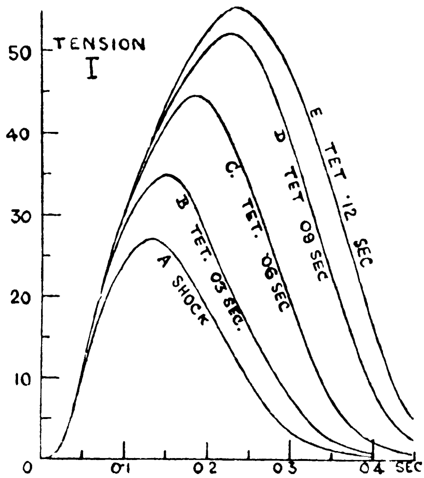

FIG. 57. Isometric Contraction Tetani showing continued rise of tension with prolongation of the excitation.

When the nerve is stimulated with a strong shock, it causes a larger contraction than if excited by a weak stimulus This is an example of another type of summation called "*multi-fibre*" or *quantal* summation, which is due to an increase in the number of motor units responding, each fibre giving an all-or-none contraction. Thus in submaximal responses, as the strength of stimulation is increased, more and more fibres respond (synchronously). Or, again, when a mixed muscle containing fibres of different contraction durations (*e g.* red and white fibres) is stimulated a summated contraction combining the features of both will result. Quantal summation is algebraical in character.

REFRACTORY PERIOD. If the interval between two stimuli sent into a curarised muscle be successively shortened in a series of observations, we finally arrive at a point at which summation of contractions is no longer apparent, *i.e.* the effect of the two stimuli is no greater than the effect of a single stimulus. This means that for a *very* short period of time after stimulation a muscle is inexcitable to a second stimulus. The period during which it is inexcitable is known as the refractory period and amounts in frog's skeletal muscle to about 3 msec. It is much shorter in mammalian muscle, and in all types is greatly increased by reduction of temperature.† The same phenomenon is a common property of excitable tissues generally.

THE TONUS OF MUSCLE

The muscles in the normal body differ from excised muscles in being frequently in a condition of slight tension, which is called tone or tonus.

* CLARK. *Journ. Physiol*, 1930, **70**, 18P; CREED *et al.* Reflex Activity of the Spinal Cord. (Oxford.) 1932.

† BAZETT. *Journ. Physiol.*, 1907, **36**, 414, KEITH LUCAS. *Journ. Physiol.*, 1910, **39**, 331.

This is due to reflex activity, since it is lost if the spinal cord is destroyed or if the motor nerves to the muscles are severed: the denervated muscle is toneless and inert.

If the tendon of a muscle *in situ* be cut and pulled, so as to stretch the muscle, the muscle pulls back against the extending force, *i.e.* its state of tonus, or tonic contraction is increased. When there is no pull upon its tendon there is little tonus, but as soon as the tendon is pulled upon the tone augments and increases with increase of the tension. This is called the "*stretch-reflex*" and is more fully dealt with under the central nervous system. If the motor nerve is cut the tonus is abolished, so it is obviously a state transmitted to the muscle through its motor nerve. But it can be shown that the slight constant contraction of tonus is not due to a slight continued shortening of all the muscle-fibres, but rather to their working, a few motor units at a time, in relays. For this reason, tonus is not followed by fatigue and may be, and is, kept up steadily for hours on end if the conditions are appropriate.

Muscle tonus is also lost if the sensory nerves which pass from the muscle and its tendon to the central nervous system are divided, even though the motor nerves remain intact. It follows from this that the reflex involved in the tonus arises in the muscle itself. The muscle is supplied with sensory organs in the shape of the muscle spindles, and the nerve endings in the tendons, and when the muscle is pulled upon these originate a stream of nervous messages which passes to the spinal cord and there initiates the discharge along the motor nerves to which the tonus is due: the stronger the pull upon the muscle the more frequent the nerve impulses which are sent from it.

Tonus is of great importance in all our muscular responses; our posture is largely maintained by its action, and all our voluntary movements take place on a background of general tonus. In diseased conditions where it is lost, the clumsy movements bear witness to the disability occasioned by its absence.

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VOLUNTARY CONTRACTION

Under normal circumstances the contraction of skeletal muscles is brought about either reflexly, or in response to some stimulus descending from the cerebral cortex, the so-called 'voluntary contraction.' These contractions may have a duration of almost any extent from about 0·1 sec., but nearly all voluntary contractions last considerably longer than that.

Since we know no certain means of extending contractions to any given length except by means of summation, it is natural that physiologists have regarded voluntary contractions as similar to the artificial tetanus produced by a summation of effect, either as wave summation or as quantal summation. In the belief that the response of each muscle fibre is invariably all or none in character, it must be supposed that the delicate gradation shown in the power of voluntary contractions is due to the participation of a variable number of motor units, the maximal force being exerted if and when all the fibres contract. Smoothness of contraction could be ensured by either mode of summation. Thus by a wave summation the same set of fibres would remain in a state of tetanic contraction throughout, whereas in quantal summation different sets of fibres would work "in shifts," each fibre giving a short tetanus or even a single twitch.

It was formerly supposed, in the belief that the response was synchronous in all fibres, that definite information as to the nature of voluntary contraction would be obtainable by a study of the electrical variations or of the mechanical records; but the accumulated evidence of quantal summation of asynchronous contractions renders such investigations of doubtful value unless single fibres, or at most only a few fibres, could be studied.

The impulse, which normally travels down the motor nerve from the anterior cornual cell to the muscle, is discontinuous. Gasser and Newcomer * have investigated the electrical changes accompanying contraction of the diaphragm, and also those in the phrenic nerve. They find that each contraction of the muscle is accompanied by the discharge, in both nerve and muscle, of about seventy separate electrical waves per second. Adrian and Cooper † have shown that interrupted stimuli at up to 230 per second can be transmitted through the spinal cord and give rise to an identical rhythm in the muscles responding reflexly to the stimulation. This evidence is in favour of the view that the stimuli causing voluntary contraction and, one must add, the tonic contractions of all skeletal muscles are discontinuous in nature and often analogous

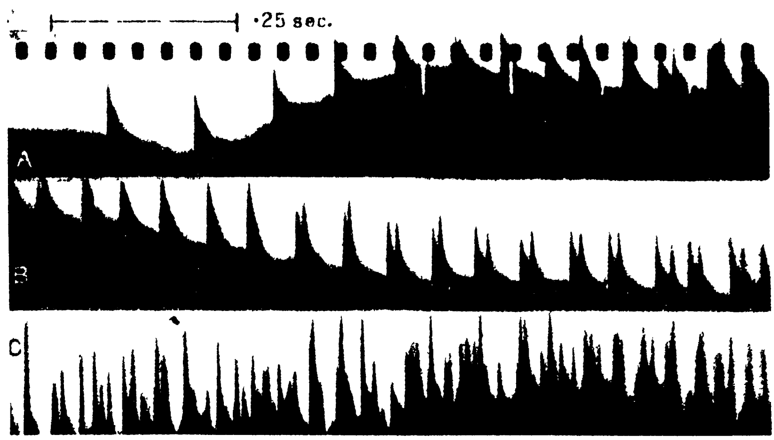

FIG. 58. Action currents in human triceps. A, weak contraction, continued at B, and showing there the entry of a new rhythm due to a fresh motor unit being excited at the same slow rate. C, powerful contraction, showing rapid rhythm of stimuli. (ADRIAN and BRONK.)

to those which we may evoke artificially by rapid stimulation either of muscle or of its motor nerve.

The problem has been solved by the work of Adrian and Bronk,‡ by the use of electrodes so small as to pick up the electrical changes accompanying contraction in only a few fibres. The electrode was made in the form of a needle which could be stuck into the muscle of a human subject. The tracings showed the arrival of stimuli at only one or two motor units. It was found that on executing a weak contraction the muscle showed a series of regular electrical changes, each of which means the arrival at one of the motor units, of a stimulus *via* the motor nerve fibre (Fig. 58, A). This stream of stimuli may be as slow as 6 per second, which would give little opportunity for wave summation, so we must suppose the summation in such weak stimuli to be quantal, and the smooth contraction of the whole muscle to be due to the fact that the various motor units involved are not contracting synchronously.

* GASSER and NEWCOMER. *Amer. Journ. Physiol.*, 1921, **58**, 1.
† ADRIAN and COOPER. *Journ. Physiol*, 1924, **59**, 61.
‡ ADRIAN and BRONK. *Journ. Physiol.* 1929, **67**, 119.

When the contraction is more powerful, fresh motor units are brought into play, and new rhythms are seen; also the rate of stimulation is increased in each motor unit (Fig. 58, B, C), so that tetanic contractions occur. We may conclude, then, that both types of summation are concerned in voluntary contractions—in weak ones, quantal summation only, in strong ones both forms.

THE MECHANICAL ENERGY OF CONTRACTION

Whereas resting muscle is soft, flabby and presents little resistance to stretching, the contracted muscle is hard, rigid and resists extension. It might be thought, therefore, that the essential feature in muscular activity consisted in a change in its elastic qualities, and that contraction involved the setting up of tension which could be used for doing work or, as in the isometric twitch, degenerating into heat as the contractile state passed away. The potential energy of a muscle in a state of isometric contraction is given by the expression $Tl/8$, where l is the length of the individual fibres

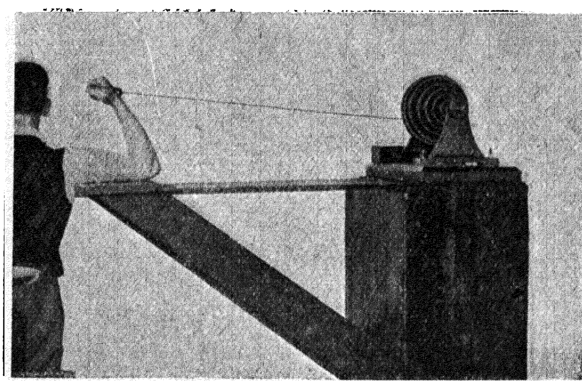

FIG. 59. Inertia flywheel for human muscle. The work done is calculated from the speed of rotation imparted to the flywheel, which weighs 35 kg, and, according to the pulley used, gives an effective mass of inertial resistance varying from 11.3 to 579 kg. (A. V. HILL. *J. Physiol.*, 1922, **56**, 19.)

and T is the tension produced.* If a stimulated muscle were simply an elastic body like a spring under tension, the amount of work which it could do when suddenly released would be a constant amount and equal to the expression just mentioned.

It is found that under most circumstances the muscle cannot realise this maximum theoretical work, but tends to do so more nearly the slower the rate of shortening. The best method of testing the power of a muscle to do work is to hinder its contraction by opposing it to the inertia of a mass, the mass being either a heavy flywheel † (Fig. 59) or an equilibrated beam.‡ If a spring under tension were made to pull against such an apparatus, the amount of work it would do would be independent of the mass moved. In muscle, however, it is found that the amount of work done against a large mass is greater than that done against a small one. The essential factor involved here is the speed with which a muscle is allowed to shorten: the

* The value $Tl/8$ is now considered more correct than that originally given, which was $Tl/6$. HILL. *Journ. Physiol.*, 1913, **46**, 435; DOI. *J. Physiol.*, 1920, **54**, 335.

† HILL. *Journ. Physiol.*, 1922, **56**, 19.

‡ HILL. *Journ. Physiol.*, 1920, **53**, *Proc.*, lxxxviii.; GASSER and HILL. *Proc. Roy. Soc.*, 1924, 96B, 398.

greater the speed of shortening, the less, within limits, will be the work done.* In the case of human arm muscles the work does not approach its maximum value unless the contraction has been opposed by a mass large enough to make it occupy at least two seconds (Fig. 60). The same relation is found in frog's muscle, the only difference being that the frog's muscle is ten times quicker than that of man.

OPTIMAL RATE OF CONTRACTION. We have seen above that the slower the speed of shortening of a muscle the greater is the amount of work done. It must be remembered however that energy is required in order to *maintain* the contracted condition in muscle.† There will thus be a point at which the extra energy required to maintain the contraction more than compensates for the gain in work done by increased duration of the contraction, so that for each group of muscles there will be an optimal rate at which the muscles will work with the greatest economy. We have seen that in the case of the human arm muscles the rate is ten times slower than in the

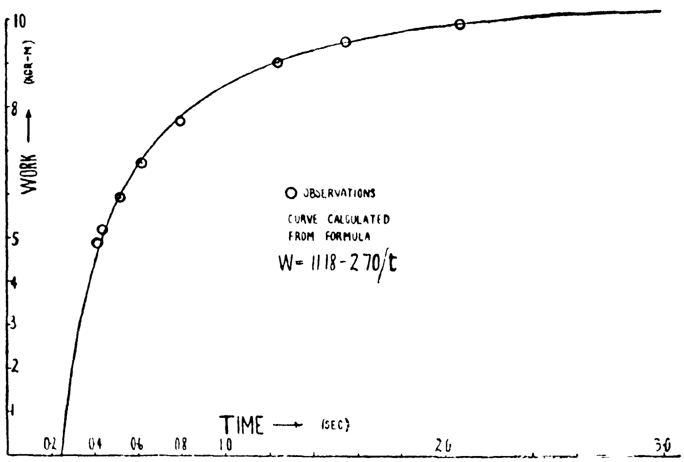

FIG. 60. Relation between maximum realisable work and duration of contraction for human arm muscles Theoretical maximal work = 11 18 kg-m (A. V. HILL. *J Physiol*, 1922, **56**, 19)

isolated frog's muscle, and probably the rate will vary according to the mass of the limb involved and therefore, to a certain extent, to the size of the individual. It is a familiar experience that the effort of going upstairs becomes less as the rate is diminished, but not indefinitely, and that it may become more tiring if the rate is made too slow. The same thing applies to practically any form of exercise in which external work is being done.

EFFECT OF INITIAL LENGTH OF FIBRE. The tension developed in a muscle contracting isometrically in response either to a single stimulus or to a short tetanus is not constant but is found to vary with the initial length of the muscle. As the length of the resting muscle is increased by extending it, up to a length somewhat greater than that which it possesses in its normal relations in the body, the tension developed on contraction also increases.‡

In the body the bony levers are so arranged that the muscles at their greatest length work at a maximum mechanical disadvantage which lessens continuously as the muscles

* HILL. *Journ. Physiol.*, 1922, **56**, 19; LEVIN and WYMAN. *Proc. Roy. Soc.*, 1927, **101B**, 218.

† BRONK. *Journ. Physiol.*, 1930, **69**, 306.

‡ EVANS and HILL. *Journ. Physiol.*, 1914, **49**, 10; DOI. *Journ. Physiol.*, 1921, **55**, 38.

shorten and approximate their points of attachment. The load on a muscle is thus diminished continuously as the muscle contracts.

The relationship between initial length and the tension developed is shown in Fig. 61. Moreover, as Lucas and A. V. Hill have shown, not only

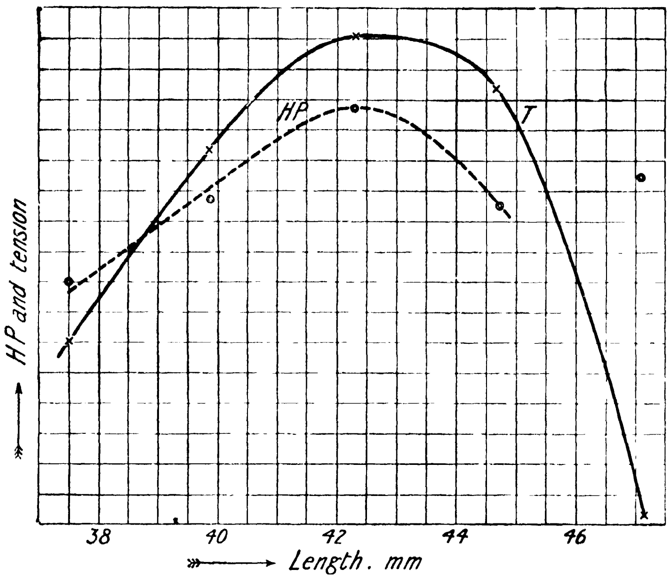

FIG. 61. Relations of heat and tension production on isometric contraction to initial length of muscle. (After EVANS and HILL, *Journ. Physiol.*, **49**, 10.)

does increased initial tension, which, of course, would increase the length of the muscle, augment the tension set up during excitation, but it also prolongs the duration of the contractile stress.

VISCOSITY CHANGES. Most of these results suggest that the change

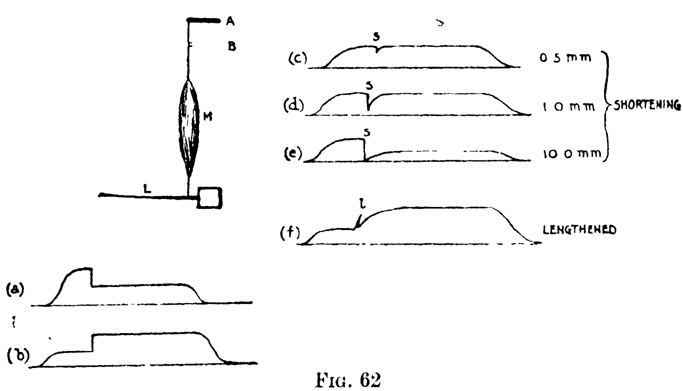

FIG. 62

occurring in a muscle on excitation involves not only a change of tension but also a change in the viscosity and elasticity of the muscle fibre.

This double change in the contracting muscle is brought out very clearly by some experiments by Gasser and Hill * on the results of a sudden partial release of the muscle undergoing isometric contraction. The apparatus used is shown diagrammatically in Fig. 62. By this arrangement a muscle M (Fig. 62), fixed at A, was tetanised and allowed

* GASSER and HILL. *Proc. Roy. Soc.*, 1924, **96B**, 398.

to contract isometrically against the spring lever L, so that at first there was no shortening; then, after a given interval, the tetanic contraction being still maintained, the end of the muscle fixed to A was suddenly released by an electromagnetic device, so that the fixed point moved down to B at a pre-arranged distance towards the lever. The muscle thus first develops its maximal tension, and then is allowed to shorten at a rate depending on the distance of B from A. In other experiments the converse was done, *i.e.* the muscle after having developed its tension was stretched over a given distance.

If the muscle behaved merely as an elastic structure, then on releasing the muscle after the maximum tension had been reached, so that it could now shorten from A to B, the tension would merely drop to a level corresponding to the reduced length as in Fig. 62 (*a*); conversely, if the muscle were suddenly pulled from A to B there should be merely an instantaneous rise in the recorded tension as in (*b*). Neither of these things actually happened, but instead, even if the shortening were very small, the tension first fell considerably (*c*) and (*d*), even to zero (*e*), and then *slowly* rose to the proper level. Similarly lengthening, after a preliminary rise, led to a slow rise to a final plateau (*f*). These curves can be approximately imitated on a thin-walled rubber tube which is filled with a highly viscous fluid. Results which are essentially similar are obtained if a stimulated muscle is stretched, or allowed to shorten, at a constant rate.* We must therefore assume that when a muscle is stimulated two changes occur: (1) a sudden rise of tension; (2) a sudden increase in the viscosity of the contents of the fibres. The result is to delay both the changes in form and the changes in tension of the contracting muscle. If it were not for this increase in viscosity, the contraction on stimulation might begin at the moment of stimulation itself, though it would always take a certain time to rise to its maximum. This double internal change in muscle is shown diagrammatically in Fig. 63. The unbroken line represents the sudden rise of tension and of viscosity beginning at the moment of stimulation. The external response, shown by the dotted line, will lag behind the internal change owing to the retarding effect of the viscosity change (see p. 164, however).

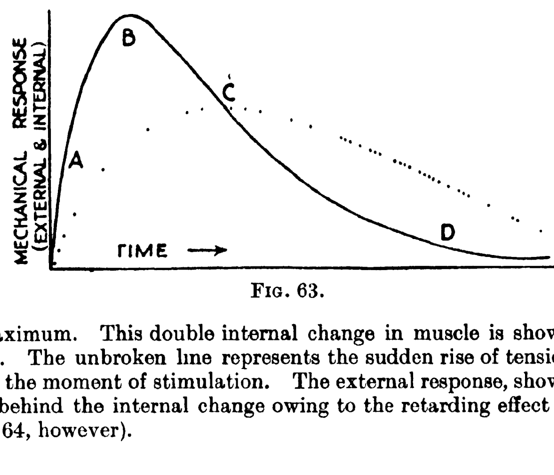

FIG. 63.

FATIGUE

A muscle will not go on contracting indefinitely. If it be repeatedly stimulated, changes soon become apparent in the curve of contraction. The latent period is prolonged, as well as the length of the contractions; the absolute height and work done are diminished. At the same time the muscle does not return to its original length; the shortening which remains is spoken of as '*contraction remainder*.' After an initial rise during the first few contractions, these diminish uniformly in height till they are no longer apparent, so that the muscle is now said to have lost its excitability. At the same time there is a great prolongation of the relaxation, so that after forty or fifty contractions several seconds may elapse before the lever returns to the base line (Fig. 64).

If left to itself, the muscle which has been exhausted by repeated stimulation will partially recover. The recovery is hastened by passing a stream of blood, or even of salt solution, through the blood vessels of the muscle. Still more important is a free supply of oxygen.

The phenomena of fatigue in isolated muscle depend on two factors:

* LEVIN and WYMAN. *Proc. Roy. Soc.*, 1927, **101B**, 218.

(1) The consumption of the substances available for the supply of potential energy to the contractile material.

(2) The accumulation of products of the contractile process. Among these waste products the lactic acid is of greatest importance. Fatigue may be artificially removed by washing out the muscle with normal saline solution containing a small percentage of alkali. The beneficial effects of oxygen supply are also due to the removal of lactic acid.

In speaking of fatigue it is important to specify the tissue which is fatigued. If instead of being stimulated directly the muscle is repeatedly made to contract by stimulation of its motor nerve, fatigue sets in much sooner; yet if the muscle be now excited directly it is seen not to be fatigued. This shows that either the nerve trunk is fatigued sooner than the muscle, or else that for some reason the muscle ceases to respond to nervous impulses sooner than to direct excitation. Actually, the latter explanation is the correct one, since nerve trunks are practically unfatiguable.

Fatigue in the intact animal as a result of voluntary contractions is not strictly comparable with that of excised muscle stimulated either directly or

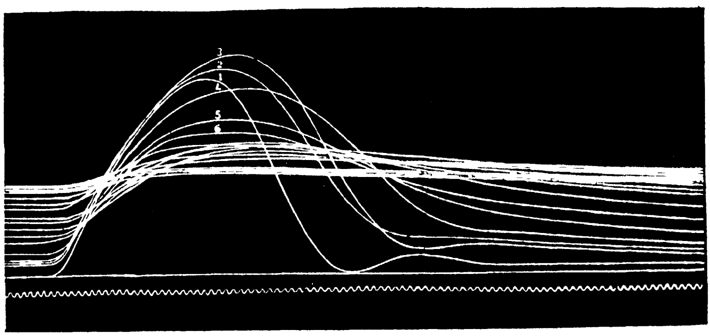

FIG. 64. Muscle Curves showing Fatigue in consequence of Repeated Stimulation. The first six contractions are numbered, and show the initial increase of the first three contractions. (BRODIE.)

indirectly. It may be studied in man by the use of instruments called ergographs, in which a finger, or one of the limbs, pulls up a weight or contracts against a spring, the degree of shortening or tension change being recorded. When after repeated contractions, fatigue has set in, the resulting tracing is very similar in appearance to that obtained from an isolated muscle stimulated electrically to the point of fatigue. But, in point of fact, in voluntary contraction the muscle is very far from being fatigued, as may be shown by applying electrical stimuli through the skin to it, or to its nerve, when powerful contractions result. What have been fatigued as the result of the voluntary efforts are the motor nerve cells responsible for the activation of the muscle.

We may conclude then, that the most readily fatiguable point is the motor nerve cell, next the conduction from nerve trunk to muscle, then the muscle, and last of all the nerve fibre.

CONTRACTURE

Skeletal muscle may, under certain conditions, enter into a state of prolonged reversible contraction which is distinct from tetanus, and which is

called *contracture.* Thus, if a fresh frog's muscle be stimulated by repeated supra-maximal opening induction shocks, the relaxation after the twitches is slow and incomplete; it is due to a prolonged action of the stimulus.* After many contractions also, a muscle shows another form of contracture, the "contraction remainder," due to delay in the relaxation process. Contractures of various types can also be produced by thermal, mechanical or chemical stimuli of the most diverse description. Contractures differ from tetani and other forms of contraction in that the contractile process is not conducted through the tissue and no wave-like action potentials occur; contractures of long duration may therefore be quite definitely localised in a muscle, the remainder of which is in a relaxed condition.

To what extent contractures may occur in the intact body is a matter of dispute; many pathological shortenings of muscle which are seen clinically and which are sometimes called contractures, are often manifestations of tonic reflexes, fibrosis, and other phenomena not connected with real contractures. But a true prolonged contracture, called "idiomuscular contraction," is seen on mechanical stimulation of certain irritable muscles.

An interesting instance of contracture is seen in muscles after section and degeneration of their motor nerves. In such muscles, stimulation of the *vaso-dilator* nerve (or of the sensory nerve) to the muscle causes a prolonged contracture. This, which is sometimes called the Vulpian-Heidenhain-Sherrington phenomenon, is of great theoretical interest. It was discovered by Vulpian and Philippeaux in 1863 that, some days after section of the hypoglossal nerve (motor to the tongue), stimulation of the chorda tympani fibres in the lingual nerve (vaso-dilator to the tongue) caused contracture of the tongue muscles. In the normal state, stimulation of the chorda tympani produced no effect at all on the tongue muscles. It was shown by Sherrington † that the phenomenon is obtainable under comparable conditions from any muscle.

The key to this apparent paradox is to be found in the theory that the action of vaso-dilator nerves is due, at least in part, to the liberation at their terminations in the tissues, of acetyl choline.‡ It has been shown that acetyl choline is a normal constituent of the muscle and other tissues.§ The muscle when deprived of its motor nerve supply is thrown into contracture by acetyl choline, as may easily be demonstrated, whereas the normally innervated muscle is less sensitive to its action. By injection of acetyl choline in large doses (*e.g.*, 1-2 μg.) direct into the arterial blood stream to a normal muscle, however, a quick contraction, followed by a slow contracture, results.¶ The effect of acetyl choline on the denervated muscle, in accordance with this hypothesis, is enhanced by eserine and abolished by atropine. What happens under the conditions of the experiment, therefore, is that when the vaso-dilator nerve is stimulated, acetyl choline is liberated in the region to which the nerve is distributed, *i.e.* in the muscle. This substance causes dilatation of the bloodvessels there, both in the normal muscle and in the denervated muscle. But in the denervated muscle the acetyl choline also produces the effect of contracture, which it does not do in the less sensitive normal muscle.

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THE ACTION OF CHEMICAL SUBSTANCES

SALTS. The action of sodium salts on muscle is of considerable interest. A 0·65 per cent. NaCl solution has a distinctly excitatory effect upon the muscle, so that single induction shocks may cause contracture. The same

* BREMER. *J. Physiol.*, 1932, **76**, 65.
† SHERRINGTON. *J. Physiol.*, 1894, **17**, 252.
‡ DALE and GADDUM. *J. Physiol.*, 1930, **70**, 109.
§ DALE and DUDLEY. *J. Physiol.*, 1929, **68** 97; PLATTNER and KRANNICH. *Pflüger's Arch.*, 1932, **229**, 730; **230**, 356.
¶ BROWN. *J. Physiol.*, 1937, **89**, 438.

excitatory effect is still better marked with solutions of sodium carbonate or oxalate which reduce the free $Ca^{\cdot\cdot}$ of the muscle. If a thin muscle, such as a frog's sartorious, be immersed in a solution containing 0·5 per cent. NaCl, 0·2 per cent. Na_2HPO_4, and 0·04 per cent. Na_2CO_3 (Biedermann's fluid), the muscle enters into a series of frequent contractions, so that it may wriggle from side to side, or may even 'beat' for a time with regularity.

This excitatory action of sodium salts is neutralised by the addition of traces of calcium salts. Hence the saline used in the laboratory should always be made with tap water, containing calcium salts. But it is better to use Ringer's solution.

Potassium salts if injected direct into the artery supplying a muscle in doses of a few mg. cause a brief twitch.* Yet if a muscle is soaked in a solution of KCl, it quickly loses its irritability. If a muscle be transfused with suitable fluids containing small amounts of potassium salts, it at once shows all the signs of fatigue, signs which may be removed by washing out the potassium salts by means of 0·65 per cent. NaCl solution. It is possible that the setting free of potassium salts may be one of the factors involved in the development of the normal fatigue of the muscle.

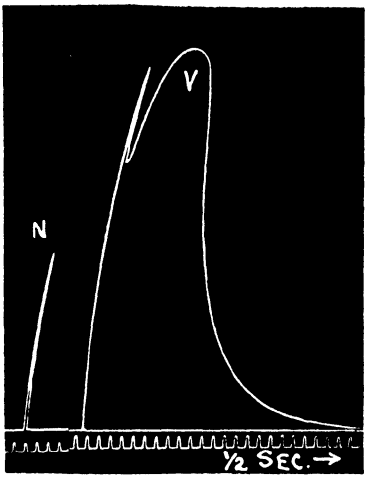


FIG. 65. Contractions of frog's sartorius. N, before, and V, twenty minutes after, soaking in Ringer's solution containing 1 : 100,000 veratrine. The records were taken on a slowly moving surface, as the time tracing shows.

If a frog's muscle be isolated and kept moist for a few hours, it often shows a complete loss of excitability, but regains it after a short immersion in Ringer's fluid, and does not become non-irritable again during its survival. The loss of excitability is apparently due to the passage of potassium salts from the inside to the outside of the fibre.†

OTHER DRUGS. Of the drugs that have a direct action on muscle, the most remarkable is veratrine. If a muscle is soaked in a 1 : 100,000 solution of this alkaloid, a single stimulus results, not in a short twitch, but in a sustained contracture, which may last for many seconds (Fig. 65). The effect appears to be due to an abnormal increase in the duration of the excitatory state. Eserine has similar effects. The heat-production of the muscle is also prolonged.‡ For some time after the contracture has died down the effect of stimulation is to give an ordinary twitch. Formaldehyde, barium salts, and many other substances produce similar contractures.§

Quinine increases the response to a single maximal stimulus, but also increases the refractory period, so that response to a tetanus is diminished. Eserine and veratrine do not produce their effects after quinine.||

Chloroform and caffeine cause the muscle to shorten and quickly to pass into *rigor mortis.* Barium salts have a similar though less marked effect.

CHEMICAL COMPOSITION OF SKELETAL MUSCLE

Muscle contains about 75 per cent. of water. The chief solid constituents are the proteins (20 per cent.) which form the bulk of the cellular structure

* BROWN. *J. Physiol.*, 1937, **91**, 4.P.
† HORTON. *J. Physiol.*, 1930, **70**, 389.
‡ HARTREE and HILL. *J. Physiol*, 1922, **56**, 294.
§ GASSER. *Physiol. Rev.*, 1930, **10**, 35.
|| HARVEY. *J. Physiol.* 1939, **95**, 45.

of the tissue. Of the remaining solids, several constituents referred to later are of great importance, though only present in small amounts.

MUSCLE PROTEINS. The contents of the living fibres, once thought to be fluid, are now recognised to be in the state of a weak gel; this is shown by their behaviour when expressed by micro-dissection operations, and also by a consideration of certain physical properties of the muscle. By mincing the living muscle with ice and a little salt and subsequently filtering under pressure, a viscous fluid formerly called "muscle plasma" can be obtained. This is not, as once thought, the unaltered cell contents, but a partial solution of the gel in dilute salt solution. If the temperature of the extract rises, it clots to a firm gel.

The older investigations of Halliburton and others have been extended, * † and it appears that the living muscle contains four proteins, called respectively *myogen, myosin, globulin X* and *stroma proteins*.

Myogen, an albumin, is obtained by dialysing the "plasma," which precipitates the myosin, and subsequently filtering. It is soluble in water and has an isoelectric point of pH 6·3, but in presence of salts this moves to the acid side. It is readily denatured at the isoelectric point. It represents about 20 per cent. of the total proteins.

Myosin, a globulin, is obtained by precipitation from a slightly alkaline (pH 7·2) saline extract by neutralisation and dilution. The precipitate is redissolved in dilute salt solution at pH 7·0.‡ Its solutions are extremely viscous and it readily forms a gel when the salt concentration is low. Its isoelectric point is dependent on the salt concentration, but is about pH 5·1. The globulin is insoluble in water, and, between pH 5 and 6, also insoluble in salt solutions. It is completely precipitated in 0·04 m. KCl at pH 7·0. About 40 per cent. of the total muscle protein is myosin, and this is largely present in the Q-discs. Here, owing to the rod-like nature of its molecules, it confers on the muscle its elasticity, its thermo-elastic properties and the known relationship between damped and undamped elasticity.

Globulin X, only slightly soluble, is completely precipitated in 0·005 m. KCl at pH 7·0. It represents about 20 per cent. of total protein.

Stroma proteins are insoluble even in concentrated salt solutions.

It is interesting to note that the amount of protein capable of extraction from muscle by means of salt solution is greater (90 per cent.) when the muscle is quite fresh. When rigor has set in only 30 per cent. of the total protein is thus extractable and § it would seem that the processes of fatigue and rigor, like that of the coagulation of fresh extracts, is the result of a process of denaturation which takes place in the protein before or after death. The hydrogen ion concentration of fresh resting muscle is probably about pH 7·0 ‖ so that the muscle proteins would be on the alkaline side of their isoelectric point, *i.e.* they would be present as salts of bases and be ionised as anions. Although coagulation can be produced in a fresh muscle by immersion in acid, it appears to be different from true rigor mortis, which, moreover, is not necessarily associated with an acidification of the muscle.

OTHER CONSTITUENTS OF MUSCLE. Of the remaining constituents of fresh resting muscle the most important are glycogen, lactic acid, and three phosphorus compounds, viz. creatine phosphoric acid, hexose phosphate and adenyl pyrophosphoric acid; all of them appear to participate in the chemical changes which are associated with contraction.

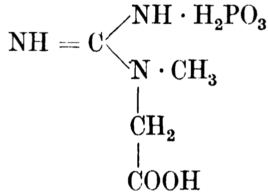
Glycogen is present up to 0·5 to 1 per cent.; embryonic muscle contains more.

Lactic acid (CH_3, CHOH, COOH) is the dextrorotatory isomer. It is only present in amounts of the order of 0·02 per cent. when muscle is fresh, well

* SMITH. *Proc. Roy. Soc.*, 1930, **105B**, 579; 1937, **124B**, 136.
† WEBER. *Ergebn. d. Physiol.*, 1934, **36**, 109.
‡ EDSALL. *J. Biol. Chem.*, 1930, **89**, 289.
§ SAXL. *Beitr. chem. Physiol. u. Path.*, 1907, **9**, 1.
‖ FURUSAWA and KERRIDGE. *Journ. Physiol.*, 1927, **63**, 33.

oxygenated and resting. When the muscle is injured and under various other conditions, glycogen is rapidly converted into lactic acid.

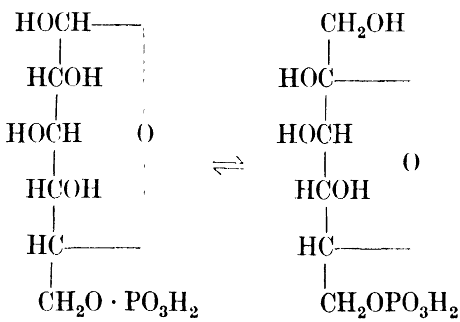
Creatine phosphoric acid. Creatine is present in amounts up to 0·5 per cent. In the fresh state, however, it is not free, but combined with phosphoric acid to form creatine phosphoric acid, or phosphocreatine, a compound to which the name of "phosphagen" was given by Eggleton.*



Creatine phosphoric acid.

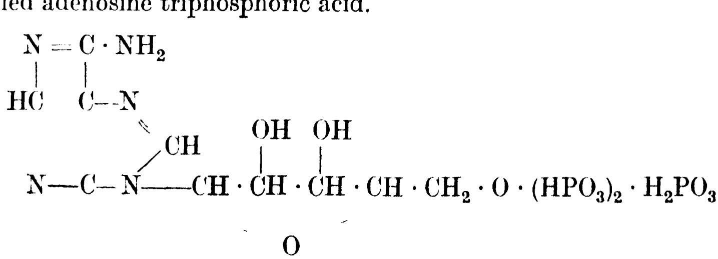
In invertebrates it is replaced by arginine phosphoric acid, which has similar functions. Creatine phosphoric acid is very readily broken down by acid, or on injury of the muscle, into creatine and phosphoric acid. It has been synthesised.†

Hexose-phosphate. Of the many possible phosphate derivatives of hexose, the one normally present is the so-called "Embden ester." It is really an equilibrium mixture of 70 per cent. of "Robison ester" and 30 per cent. of "Neuberg ester."



"Robison Ester." *"Neuberg Ester"*

Adenyl pyrophosphoric acid. The presence of pyrophosphate in muscle was discovered by Lohmann,‡ who subsequently found that it was present in combination with the nucleotide adenylic acid, which is itself a compound of *o*-phosphoric acid and the nucleoside adenosine. The adenyl pyrophosphate thus contains three atoms of phosphorus, and is sometimes called adenosine triphosphoric acid.



Adenyl Pyrophosphate.

* EGGLETON. *Physiol. Rev.*, 1929, **9**, 432.
† ZEILE and FAWAZ. *Ztschr. f. Physiol. Chem*, 1938, **256**, 193.
‡ LOHMANN. *Bioch. Ztschr.*, 1928, **202**, 466.

It readily breaks up, splitting off the pyrophosphate which is then converted into two molecules of o-phosphate. Hypoxanthine, which is present in muscle extracts, is chiefly derived from the adenylic acid of the tissue, and only to a small extent from the nuclein of the cell nuclei.

Other constituents are :—

(a) *Colouring Matter.* The red muscles contain an iron-holding pigment which closely resembles hæmoglobin ; it has been isolated in crystalline form* and called *myoglobin,* or myo-hæmoglobin. It probably acts as a temporary oxygen store for the muscle. In addition all skeletal muscle contains a pigment, *cytochrome* † also related to hæmoglobin, which probably has important functions as an oxidation catalyser.

(b) *Carnosine* is a dipeptide, β-alanyl histidine. It is present to about 0 3 per cent., but its functions are unknown. In some animals it is replaced by a methyl derivative called anserine.

(c) *Inositol* ($C_6H_{12}O_6 \cdot 2H_2O$) is not a carbohydrate, but hexahydroxybenzene ; its significance is unknown.

(d) *Fat, Lecithin and Cholesterol* in traces.

(e) *Inorganic Constituents.* The ash of muscle forms from 1 to 1 5 per cent. and consists chiefly of potassium phosphate, with traces of Ca, Na, Mg and Fe in the fresh tissues. Most of the bases are normally combined with the proteins, while the phosphate is present in the various organic forms mentioned above. We therefore find that the phosphate does not diffuse out from the living resting muscle, though it does so to some extent after stimulation. This, as we shall see later, is because of its temporary liberation from organic combination.

(f) *Enzymes.* Various enzymes have been prepared from muscle and probably play an important part in bringing about the chemical changes which occur in connection with contraction ; thus there are enzymes which hydrolyse the phosphoric esters, or glycogen, or which convert glycogen into lactic acid, or which cause deamination, &c., with formation of various intermediary products.

Among dehydrogenases which probably play a part in the metabolism of muscle are those which convert :—

Lactate → Pyruvate
Malate → Oxalacetate
Triose phosphate → Glyceric acid phosphate
α-Glycerophosphate → Triose phosphate
Succinate → Fumarate
β-Hydroxybutyrate → Acetoacetate

THE CHEMICAL CHANGES WHICH ACCOMPANY CONTRACTION

The energy of the contraction of muscle must ultimately be derived from chemical changes occurring in it. In seeking out the nature of these changes three methods are open to us :

(1) We can examine the changes in the muscle either *in situ*, or surviving

(2) We can investigate the changes in the blood or in an artificial medium surrounding the muscle. Muscle may be exposed in nitrogen or oxygen in a confined space, and its gaseous interchanges during rest and activity compared. The muscle may be suspended in Ringer's solution, with or without oxygenation, or we may determine the change in the composition of the blood in passing through the muscle.

(3) We may compare the changes in the total metabolism of the animal during rest and muscular work as a clue to the muscular metabolism itself.

We may begin by dealing with the evidence afforded by the first two methods of investigation and then see how far our conclusions are borne out by experiments on the metabolism of the whole animal.

* THEORELL. *Bioch. Ztschr.*, 1932, **252,** 1.
† KEILIN. *Proc. Roy. Soc.*, 1925, **98B,** 312.

THE LACTIC ACID CYCLE IN SURVIVING MUSCLE

Formation of Lactic Acid. The lactic acid content of muscle is estimated by mincing it up as quickly as possible in an ice-cold solution of trichloracetic acid. After removal of carbohydrate from the extract the lactic acid of the filtrate is determined.

It is easy, by modern methods, to confirm the fact, originally elicited by Fletcher and Hopkins,* that fresh resting muscle contains only minimal amounts of lactic acid, provided that care is taken to avoid injury to the muscle and to keep its temperature low until it has been killed by the reagent. Even in mammalian muscle, the rate of formation of lactic acid is quite slow provided the muscle is uninjured and at rest. If the muscle has been stimulated and made to contract immediately before being killed in this way, its lactic acid content is found to be raised considerably, and if kept for a few seconds after stimulation it is higher still. The total rise in mammalian muscle may be from a resting value of 0·02 per cent. up to a final value of 0·5 per cent. These experiments prove that lactic acid is formed when a muscle contracts, and continues to be formed for a short time after the contraction is over. It is also easy to show that as the lactic acid increases the glycogen content of the muscle diminishes. If the muscle be damaged, a steady formation of lactic acid takes place, which is more rapid in the first few hours after death, but continues until the muscle passes into rigor.

With the completion of rigor, frog's muscles are found to contain about 0·4 per cent. lactic acid. After this the amount does not increase. The onset of rigor, and the rate of production of lactic acid, are quickened if the muscle be kept warm and if oxygen be excluded. It is interesting to note that the amount of lactic acid found in rigid muscle is almost invariable, whatever the previous history of the muscle. Thus, if the fresh muscle be finely minced it is found to contain about 0·2 per cent. lactic acid. If, however, it be allowed to stand after mincing, there is a slow production of lactic acid up to the maximum 0·4 per cent. Again, a muscle which has been tetanised to exhaustion contains about 0·2 per cent. lactic acid. When allowed to undergo rigor, the amount rises to about 0·4 per cent. This limitation depends on the fact that the accumulation of lactic acid inhibits its further formation. If care be taken to neutralise the acid as it is formed by immersing the muscles in weak alkaline solutions, especially of phosphates, *e.g.* Na_2HPO_4, and to exclude oxygen, the whole of the glycogen disappears and as much as 1·2 per cent. lactic acid can be obtained.

The lactic acid which is formed in muscles by their contraction is immediately neutralised. The process is complex, and so perfect that little if any change of reaction occurs until fatigue begins to set in.

In this neutralisation the inorganic salts of the muscle take but a small part, but an important *rôle* in the process is played by the buffering action of the proteins of the muscle, which exist as ionised compounds of protein and potassium or sodium—as in the following equation :

$$\underbrace{Na^+ + P^-}_{\text{(sodium proteinate)}} + \underbrace{H^+ + L^-}_{\text{(lactic acid)}} \rightarrow \underbrace{Na^+ + L^-}_{\text{(sodium lactate)}} + \underbrace{HP}_{\text{(undissociated protein)}}$$

Two points regarding lactic acid formation are important. First, the lactic acid formation does not precede or accompany a contraction but follows it. Hence it cannot be the cause of the contraction. Second, lactic

* Fletcher and Hopkins. *J. Physiol.*, 1907, **35**, 247; *Proc. Roy. Soc.*, 1917, **89B**, 444.

acid formation occurs without any oxygen utilisation and is indeed independent of the presence of oxygen.

Source of the Lactic Acid. The disappearance of glycogen as lactic acid appears points to that substance as the precursor of the lactic acid. Moreover, the maximal amount of lactic acid obtained is never greater than corresponds to the original glycogen content. The change requires the presence of phosphates, and takes place by a series of complex phosphorylated intermediary stages which are discussed in some further detail later. For the present we need only note that hexose and triose phosphates are important intermediaries.

The following schema represents the changes involved, and will be called "*the lactic acid cycle.*" It must be remembered that (as we shall see in dealing with the changes following activity) in the living muscle all these steps are reversible.

$$\begin{array}{c}\text{Glycogen}\\ \downarrow\quad\uparrow\\ \text{Hexosediphosphate}\\ \downarrow\quad\uparrow\\ \text{Triose phosphate}\\ \downarrow\quad\uparrow\\ \text{Lactic acid.}\end{array}$$

The reverse change of conversion of lactic acid into glycogen does not occur in the absence of oxygen. When oxygen is available, however, it is found that if the muscle is allowed to rest the lactic acid is removed, and glycogen returns.

THE OXYGEN USAGE OF MUSCLE. If we excise a thin muscle, *e.g.* the sartorious of a frog, and stimulate it repeatedly in an atmosphere of oxygen it ultimately becomes fatigued and incapable of further contraction. On allowing the muscle to rest, however, it recovers considerably and can execute a further series of contractions, again resulting in fatigue. The recovery is hastened by washing the muscle in oxygenated Ringer's solution.

An isolated frog's muscle can also contract equally well if placed in an atmosphere of nitrogen. If repeatedly stimulated in nitrogen fatigue

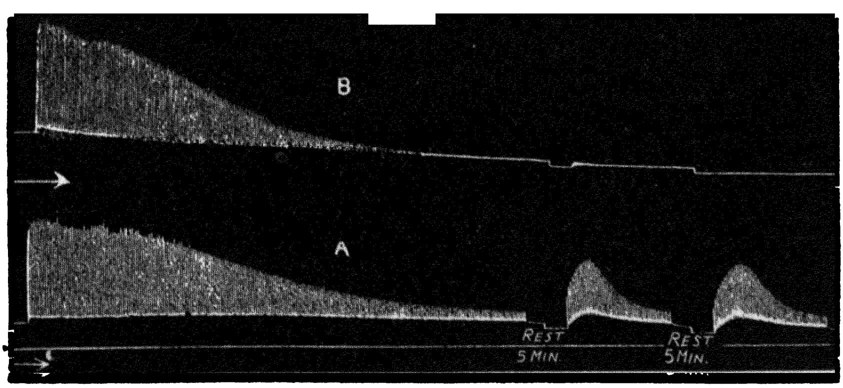


FIG. 66. Fatigue in a Pair of Sartorii. (FLETCHER.)
Max. break shocks one per second. Load 6 grammes. Temp. 19° C.
A. Exposed to oxygen. *B.* Exposed to nitrogen.

finally sets in; but on cessation of the stimulation the muscle in nitrogen does not recover, or recovers very little, and soon passes into rigor * (Fig. 66).

These facts suggest that although oxygen is not essential for contraction it is necessary for recovery. It is therefore of interest to examine the oxygen usage of excised frog's muscle.

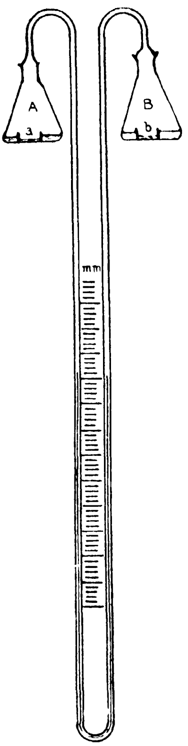


FIG 67

For the investigation of the oxygen utilisation of isolated tissues much use has been made of the *microrespirometer*,† the principle of which is illustrated by Fig. 67. The two small flasks A and B are of equal size, and are connected each with one limb of a fine-bore manometer filled with kerosene and provided with a millimetre scale. On the bottom of each of the flasks is a shallow receptacle (*a*, *b*) in which are placed a few drops of potash solution; the tissue, in a thin sheet, or minced, is floated in Ringer's or other suitable solution in the bottom of one flask, the other containing Ringer's solution only. The bottles are placed in a thermostat and filled with oxygen. The menisci of the manometer are read, and the whole apparatus is continuously agitated in a horizontal direction to and fro to keep the solution saturated with oxygen. At intervals the reading of the meniscus in each limb of the manometer is taken. The level of the kerosene ascends in the manometer on the side connected with the bottle containing the tissue, and falls on the other side. The carbon dioxide produced by the tissue is absorbed by the potash, so that the movement of the kerosene is entirely due to the oxygen absorbed, the volume of which can thus be calculated. Thus the oxygen utilisation of the tissue under given conditions can be found over any required period of time.

If a parallel experiment is made with omission of the potash from the receptacles the change of volume is due to the difference between the oxygen used and the carbon dioxide formed, and from this, if the oxygen used is known, the respiratory quotient can be calculated.

If a thin muscle be placed in oxygen in a microrespirometer, its resting oxygen usage is found to be quite small, and its R.Q. is 0·85 to 0·90.‡

When muscle is stimulated, its oxygen usage and all its chemical changes are accelerated.

If poisoned with sodium azide, NaN_3, however, although the resting oxygen is unaffected, there is no increased usage on contraction, however produced.§ Cyanide abolishes both resting and activity usages. Probably the cyanide inactivates all the enzyme systems, while azide only inactivates the cytochrome oxygen transport system. If this is so, that system must be only brought into play in contraction.

If the muscle is stimulated in nitrogen, lactic acid very quickly accumulates, and the "fatigue maximum" is soon reached. If the muscle is a thin one, *e.g.* the frog's sartorius, and it be suspended in oxygen-free Ringer's solution, the lactic acid diffuses away and fatigue does not set in so soon.

When oxygen is readmitted to a muscle which has been brought to fatigue in nitrogen, the oxygen usage is raised above the former resting value, while the "oxygen debt" contracted during the anaerobic period of stimulation is being wiped off; this corresponds with removal of lactic acid and

* FLETCHER. *J. Physiol.*, 1902, **28**, 474.
† KROGH. *Bioch. Ztschr.*, 1914, **62**, 266. There are many patterns of microrespirometer.
‡ FENN. *J. Cell. and Comp. Physiol.*, 1932, **2**, 233.
§ STANNARD. *Amer. J. Physiol.*, 1939, **126**, 196.

recovery of excitability of the muscle. This increased oxygen usage ("excess oxygen") following activity is at first high; it subsides, at first quickly, then more slowly, to the original basal level, which may only be reached after many hours. As soon as the oxygen usage has returned to the resting level, but not before, the lactic acid is also found to have the low value which characterises the resting tissue. The "excess CO_2," is found to be equal to the excess oxygen, so that the respiratory quotient of the recovery process is unity; that is to say, it suggests that the oxygen which is utilised in recovery burns up either lactic acid or carbohydrate.

But when we compare the excess oxygen usage in recovery with the *amount* of lactic acid which has disappeared, it at once becomes clear that the whole of the lactic acid cannot have been removed by oxidation, for, according to the equation

$$\underset{(90\text{ g})}{C_3H_6O_3} + \underset{(67\text{ l})}{3\ O_2} = 3\ CO_2 + 3\ H_2O$$

each mg. of lactic acid removed should require 744 c.mm. of oxygen for its oxidation, whereas under usual conditions a usage of only 149 c.mm. of oxygen per mg. of lactic acid lost is an average figure, that is, about one-fifth of the amount which would be needed for complete oxidation of the lactic acid that has gone. The fraction varies with the condition of the muscle, becoming larger the less efficient its operation.

The fate of the lactic acid which is *not* oxidised becomes clear when the glycogen content of the muscle is followed during recovery, for there is an increase in the glycogen content of the muscle which is equivalent to the amount of lactic acid removed, but not oxidised. We can thus write the following equations for the anaerobic and recovery stages concerned in the formation and subsequent removal of 1 g. of lactic acid, assuming recovery efficiency of one-fifth.

(1) $\underset{\text{Glycogen.}}{C_6H_{10}O_5} + H_2O = \underset{\text{Lactic acid.}}{2\ C_3H_6O_3}$

162 g. ⟶ 180 g.

∴ 0·9 g ⟶ 1 g. (O_2 usage missed in anaerobic period = 149 c.c.)

Anaerobic period

(2) 1 g lactic acid → 0·2 g. oxidised

$$\underset{(0\cdot2\text{ g.})}{C_3H_6O_3} + \underset{(149\text{ c.c.})}{3\ O_2} = \underset{(149\text{ c.c.})}{3\ CO_2} + 3\ H_2O$$

→ 0·8 g. converted to glycogen (0·72 g.)

$$\underset{(0\cdot8\text{ g.})}{2\ C_3H_6O_3} = \underset{(0\cdot72\text{ g.})}{C_6H_{10}O_5} + H_2O$$

Recovery period

The lactic acid cycle now becomes:—

$$\begin{array}{c} \text{glycogen} \\ \downarrow \quad \uparrow \tfrac{4}{5} \\ \text{Lactic acid} \\ \downarrow \\ \tfrac{1}{5} \text{ oxidised to } CO_2 + H_2O \end{array}$$

In an atmosphere of oxygen the effect of stimulating the muscle is to raise its oxygen usage to ten or fifteen times the resting value; if the muscle were thin enough to allow of access of oxygen to all its parts, lactic acid would not accumulate, as a state of balance would be reached, the oxidation-restitution process keeping pace with the production of lactic acid. In

experiments with excised muscles these conditions were shown by Hill and Kupalov* to be realised when frog's sartorii were suspended in oxygenated Ringer's solution and stimulated twenty to thirty times a minute. Such a muscle could give more than 10,000 twitches.

LACTIC ACID CYCLE IN THE INTACT BODY

When we turn to experiments on the whole animal (or on man) we obtain some confirmation, but also some modification, of the views as to the intimate nature of muscular activity which have been derived from experiments on isolated frogs' muscles.

The total energy of the body is derived ultimately from the oxidation of foodstuffs. The products of this oxidation are excreted partly with the urine, partly with the expired air, so that examination of these excretions, combined with a measurement of the oxygen taken into the body in respiration, serves as a measure of the total oxidations occurring in the body. Many experiments on the effect of exercise have shown that the excretion of nitrogenous substances is not immediately affected,† while on the other hand any change in the amount of muscular activity is revealed at once by a corresponding alteration in the respiratory exchanges. When a man runs he becomes 'out of breath,' and this is associated with an increased intake of oxygen and excretion of CO_2. We must conclude, therefore, that the main source of muscular energy is the oxidation of carbon: whether in the form of carbohydrate or of fat we shall have to discuss shortly.

A man at rest absorbs from the respired air about 300 c.c. oxygen per minute, and gives out about 250 c.c. CO_2. The quotient of CO_2 given out by oxygen absorbed is called the 'respiratory quotient,' and, when measured over sufficiently long periods, its magnitude depends on the nature of the substances undergoing oxidation. If these were entirely carbohydrate (or lactic acid) the respiratory quotient CO_2/O_2 would be equal to 1:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$

If fats alone were being utilised, the respiratory quotient would be 0·71; with proteins alone, it would be 0·81. In man, on a mixed diet, the respiratory quotient at rest is somewhere about 0·85.

Even the mildest form of exercise increases the intake of oxygen and output of CO_2. Thus, in one experiment, walking at 3·5 miles per hour raised the oxygen intake from the resting value of 292 c.c. per minute to 1155 c.c. per minute. With increased intensity of muscular exercise the intake of oxygen may go up largely and may rise to 3000 c.c. or in athletes to even 5000 c.c. per minute. A well-trained man can continue to run without getting further distressed while he is using 3500 c.c. oxygen per minute. On ceasing exercise the increased pulmonary ventilation rapidly falls, and after a period varying from ten minutes to half an hour the respiration and the oxygen intake have returned nearly to their previous amounts.

When the body is at rest, the circulating blood contains about 15 mg. lactic acid per 100 c.c. This originates from glucose in the circulating blood by an enzyme action, called *glycolysis*:—

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2C_3H_6O_3}$$

Glycolysis is always occurring in normal blood, but is accelerated if, as by deep breathing, the blood carbon dioxide is reduced and the blood thereby

* HILL and KUPALOV. *Proc. Roy. Soc.*, 1929, **105B**, 313.
† WILSON. *J. Physiol.*, 1932, **75**, 67

rendered more alkaline. It is also accelerated as the blood traverses the lungs, the tissue of which exerts a glycolytic action.* Lactic acid is also being removed constantly from the blood as it is formed ; a considerable amount is removed and utilised by the heart,† part may re-enter muscle and there be converted to glycogen, but the largest part of it is taken up by the liver and converted again into glycogen, probably by a process similar to that which takes place in the muscle. The glycogen formed in the liver can then be converted into blood glucose, from which the muscles seem to form glycogen more readily than from lactic acid. Hence, when at rest, the blood lactate, which represents a balance between formation and removal, remains constant.

In the living body the richness of the capillary supply, by offering oxygen freely, gives very favourable conditions for recovery, so that the rate of disappearance of lactic acid is at least eight times as fast in a muscle *in situ* as in an excised muscle. When exercise is taken, unless this be violent, there is no increase in the lactate of the blood,‡ because the muscle lactate

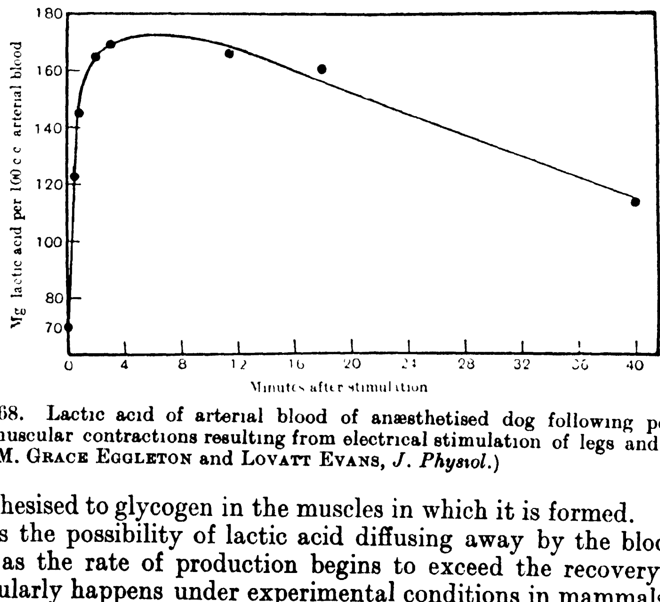


FIG. 68. Lactic acid of arterial blood of anæsthetised dog following powerful muscular contractions resulting from electrical stimulation of legs and trunk. (M. GRACE EGGLETON and LOVATT EVANS, *J. Physiol.*)

is resynthesised to glycogen in the muscles in which it is formed. But there is always the possibility of lactic acid diffusing away by the blood stream as soon as the rate of production begins to exceed the recovery velocity. This regularly happens under experimental conditions in mammals ; within half a minute after a short tetanic contraction, the venous blood coming away from the muscle contains lactic acid at a concentration almost equal to that in the muscle, and very soon the arterial lactate also reaches a high value§ (see Fig. 68). A rise of blood lactate (Fig. 73) also suggests that under normal conditions in man some of the lactic acid produced in violent contractions passes out from the muscle into the blood stream. During the subsequent recovery period the blood lactate rapidly falls again owing to removal by liver, heart and muscles and to a slight extent by excretion in the urine.

It will be seen that when the body as a whole is considered, the net result of exercise and recovery therefrom is the same as in the isolated frog muscle,

* EVANS, HSU and KOSAKA. *J. Physiol.*, 1934, **82**, 41.
† EVANS, GRANDE and HSU. *Q. J. Exp. Phys.*, 1935, **24**, 347.
‡ OWLES. *J. Physiol.*, 1930, **69**, 214.
§ EGGLETON and EVANS. *J. Physiol.*, 1930, **70**, 269.

namely, glycogen is burned, and any lactic acid formed is removed by combustion or reconversion (of four-fifths) into glycogen.

When exercise can be maintained indefinitely, the processes of recovery in the muscles are keeping pace with the processes of contraction, *i.e.* the lactic acid that is formed during contraction is being steadily removed, or oxidised and replaced as glycogen, so that there is no great accumulation of this substance either in the muscles or in the blood. Indeed, in such exercise as walking at 4·0 to 4·5 miles per hour there is no increase of blood lactate at all.

When exercise commences, lactic acid is formed at a greatly increased rate ; as soon as this happens the oxygen consumption, by which it is removed, is likewise accelerated ; as the lactic acid continues to be formed, so the oxygen intake continues to grow. Hence the more lactic acid there is formed in the muscle, the faster becomes the oxidative removal, which tends to prevent its accumulation. Ultimately (assuming the exercise to be moderate) the speed of the recovery process becomes equal to that of

FIG. 71.

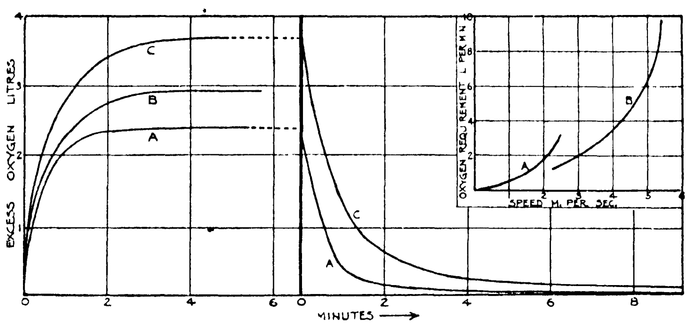


FIG. 69. FIG. 70.

FIG. 69 Excess oxygen intake at commencement of work of different degrees of severity. In A and B the steady state is reached. In C the limit of supply is reached. (After HILL and LUPTON.)
FIG. 70. Recovery excess intake of oxygen after work. A and C represent recovery from work comparable with that in the curves A and C of Fig. 69.
FIG. 71 Relation between speed of locomotion and oxygen requirement. Curve A, walking; curve B, running. (After HILL, LONG and LUPTON.)

the break-down process. This is shown by a study of the curve for oxygen intake from time to time during the exercise.* The oxygen intake per minute rises from the resting level, at first quickly, then more and more slowly, finally to reach a uniform rate which can be kept up for a considerable length of time (Fig. 69, A and B). This uniform rate is called the "*steady state*," and signifies the attainment of a steady rate of oxygen usage, together with the upkeep of a constant content of lactic acid in the muscle by balance of the rates of formation and removal. The more severe the exercise, the higher will be the oxygen usage and lactic acid content when the steady state has been reached (Fig. 69). With normal men taking such exercise as leads to an oxygen usage up to 1·8 l/min. (*i.e.* up to 6 or 7 times the resting intake), as by walking at speeds up to 4½ miles per hour, oxygen usage is adequate to ensure removal of lactate from the muscle without any escape of lactate into the blood stream. Whether there is some increase in lactic acid in the muscle, however, is uncertain.† Since, even in mild exercise there is still an

* HILL, LONG and LUPTON. *Proc. Roy. Soc.*, 1924, **96B**, 438.
† FLOCK, INGLE and BOLLMAN. *J. Biol. Chem.*, 1939, **129**, 99.

oxygen debt (Fig. 69), though often only a small one, it has been supposed by some that there is a slight increase of muscle lactate, but the matter cannot be regarded as proved.

So far we have only considered exercise which can be continued more or less indefinitely. As the severity of the exercise is increased, so the oxygen utilisation necessary to enable the steady state to be kept up will augment, and the amount of lactic acid present during the steady state will ultimately increase, some of it overflowing into the blood stream. But so long as the recovery process can ultimately catch up with the breakdown process, *i.e.* so long as the oxygen (blood) supply to the muscles is adequate, the lactic acid remains near to the level value of the steady state, so that the total amount present in muscle or blood, and the corresponding oxygen debt, are not excessive. Such exercise can be kept up for very long periods. But

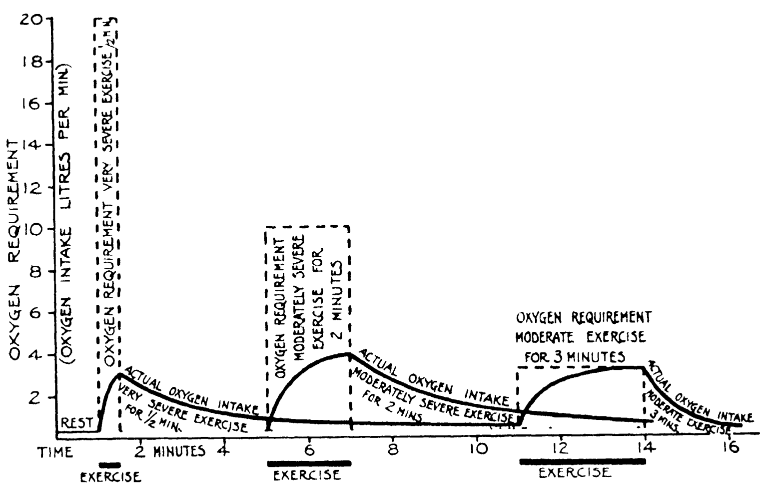


FIG. 72. Diagram to illustrate the Meaning of 'Oxygen Requirement,' and the Distinction between 'Oxygen Requirement and 'Oxygen Intake.'

The area of an oxygen requirement rectangle is, by definition, the same as that of the complete oxygen intake curve, each being measured from the level of the resting oxygen intake (shown dotted). Three periods of exercise of different severity.

if the exercise becomes really severe the oxygen supply reaches the limiting value and cannot be further increased because the heart cannot pump blood any faster; then the steady state is no longer possible, and lactic acid accumulates more and more, rises to a high level in the blood, and finally brings the performance to an end as soon as the fatigue maximum has been reached. In such cases the oxygen debt is a very heavy one, and the process of recovery is prolonged (Fig. 69, curve C, and Fig. 70, curve C).

The maximum severity of this exercise depends on the training of the individual, and physiologically on the extent to which his circulatory system and lungs can provide the working muscles with oxygen in proportion to their needs. The maximum intake of oxygen which, even in an ordinary athlete, can be carried by the blood and pumped round by the heart to the muscles is about 4000 c.c. per minute. Much severer forms of exercise are, however, possible than could be met by such an oxygen supply. In the ten or eleven seconds occupied in a 100 yards sprint, there is not time enough for the oxygen intake to be raised much above the resting level. Immediately

after completing the course the man pants violently and in the next few minutes will take in an excess of, say, 4 litres of oxygen. During these eleven seconds the man's muscles have been contracting practically anaerobically, with considerable production of lactic acid. The oxidative removal of this lactic acid has been postponed to the end of the race. The man has thus 'gone into debt' for oxygen, and the extent to which he can run into debt depends on the amount of lactic acid which can accumulate in the working muscles without stopping their activity. After any severe form of exercise this oxygen debt is apparent (Figs. 69, C; 72). The highest amount which has been observed by A. V. Hill was 19 litres; this would be equivalent to 133 grammes of lactic acid, *i.e.* if it all remained in the muscles, to nearly

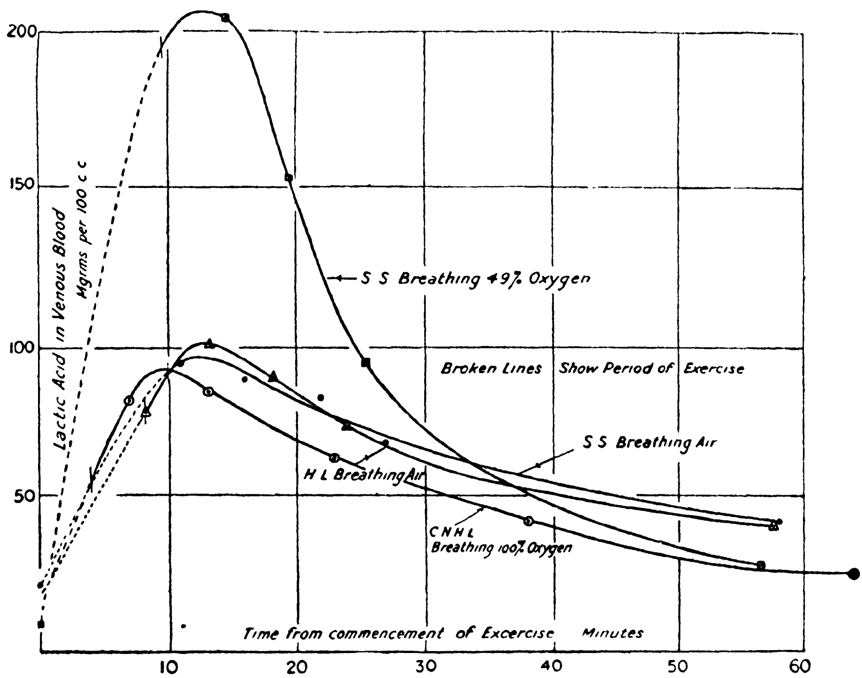


FIG. 73. Lactic Acid in Human Blood after severe Muscular Exercise. Two experiments in air—one in 49 per cent. oxygen, one in 100 per cent. oxygen. Note that the recovery process is not quite complete at the end of the time shown in the diagram. (HILL, LONG and LUPTON.)

0·4 per cent. lactic acid in the contracting muscles. A usual figure of exhaustion is about 90 grammes. The extent of the accumulation of lactate in the blood in man is shown in the accompanying diagram (Fig. 73). It will be noted that the lactic acid which has accumulated in the muscle during the severe bout of exercise continues to escape when the exercise comes to an end, so that for the first few minutes its percentage amount in the blood first rises and then falls steadily as the lactic acid is taken up by the liver and muscles and converted into glycogen again.

In spite of the apparent simplicity of the case as presented above, there are certain facts which point to its not being in reality quite so simple. Thus injection of lactate into the blood stream should cause increased oxygen usage, but does not. The calculations based on blood lactate in exercise are thus probably misleading except in so far as they provide a guide to muscle lactate concentrations. In all probability very little

blood lactate returns to the muscle, most of it being taken up by heart and liver. Hence the failure of attempts to show a close correlation between oxygen usage and fall of blood lactate after exercise.

Effect on Respiratory Quotient. Though moderate exercise, such as gentle walking, does not alter the respiratory quotient, very considerable disturbances are brought about by severe exercise. These are due to driving out of CO_2 during the exercise, followed by compensatory retention of CO_2 afterwards. We have seen that the lactic acid produced as a result of contraction is neutralised. But when much lactic acid is formed it will ultimately increase the *c*H of the blood, and therefore will stimulate the respiratory centre. Such a stimulation is necessary for the increased intake of oxygen for the supply of the working muscles, but it has also the effect of quickening the elimination of CO_2. Moreover, the ultimate escape of lactic acid into the blood will drive out carbonic acid from this fluid and will further

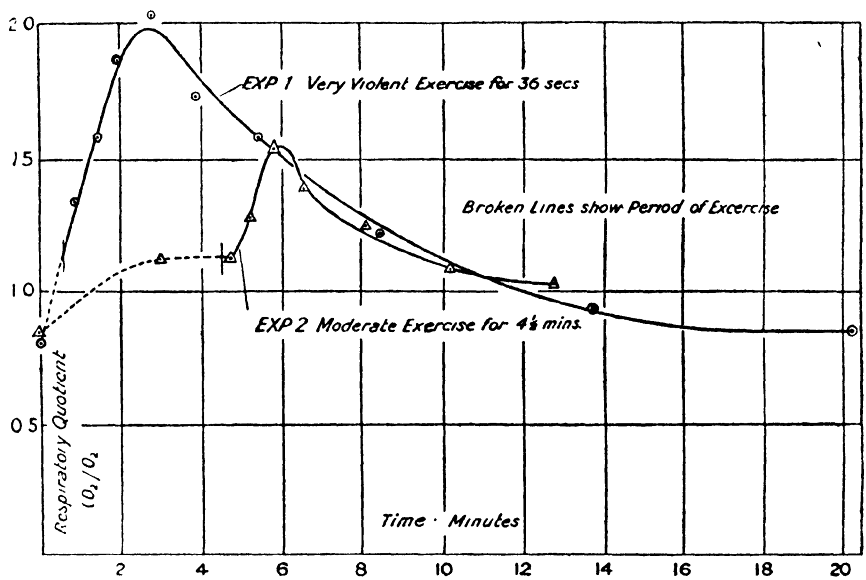


FIG. 74. The Respiratory Quotient during and after Muscular Exercise. These figures show the initial phase of recovery only. (HILL, LONG and LUPTON.)

increase its elimination by the lungs. Thus an amount of carbon dioxide would be blown off from the lungs in excess of that produced by the metabolism of the body. For example, if the true metabolism during exercise were: oxygen 3 litres per minute, CO_2 2·4 litres per minute, the true respiratory quotient would be 0·8. If, however, 3 litres extra of carbon dioxide were displaced by the entry of lactic acid into the blood and tissues, and this was eliminated in ten minutes, the apparent respiratory quotient would be raised to 0·9. This rise of CO_2 output and of respiratory quotient would be the initial event in any steady form of exercise, so that the increased breathing would be more marked while this extra CO_2 was being eliminated. After its elimination the organism enters into a steady state : the excessive breathlessness disappears, and the individual can now go on running steadily for a long time, having attained the condition known as 'second wind.' With severe exercise of short duration, when a considerable oxygen debt is incurred, the escape of lactic acid into the blood causes a great dyspnœa immediately after the exercise, with a large output of CO_2. The apparent respiratory quotient

under these circumstances may rise to 1·5 or even to 2 (*cf.* Fig. 74). During the process of recovery the lactic acid is gradually removed and the excess CO_2 which has been given off from the body is once more retained. We shall therefore expect to find that the high respiratory quotient immediately after heavy exercise is followed later on by a long lasting fall of the respiratory quotient below the resting level.

SUBSTANCES CONSUMED BY WORKING MUSCLE. For brief exercise of moderate severity it has been argued that if we make due allowance for these fluctuations in the respiratory quotient, due to the initial driving off of the CO_2 and its subsequent retention as the lactic acid disappears, we arrive at the corrected respiratory quotient of the exchanges, directly due to muscular exercise of short duration. In making this calculation it is customary to deduct the oxygen usage and CO_2 production during rest and to call the remainder the 'excess metabolism' of exercise plus recovery. One of the difficulties in this type of experiment is that of deciding how long a time is necessary for return of the respiratory exchange to the normal resting level. It has been claimed that this excess metabolism has a R.Q. of about 1·0. We are therefore perhaps entitled to conclude that in the whole animal, as in the isolated frog's muscle, the energy of muscular contraction, when this is of moderate severity, is derived ultimately from the oxidation of a carbohydrate, to CO_2 and water.

That carbohydrate, though the choice, is not the sole possible source of muscular energy, however, is indicated by several converging lines of evidence. The carbohydrate stores of the muscles can be brought to a low level by fasting, or by a diet consisting almost entirely of fat, and containing no carbohydrate. After three days of such a diet the subject is disinclined for exercise and easily susceptible to fatigue. Control experiments on animals show that under these conditions the glycogen content both of muscles and liver is lowered. Experiments by Furusawa * suggest that even after the R.Q. has been reduced to 0·75 by such a diet, the excess metabolism of a short complete cycle of exercise and recovery may have a respiratory quotient of unity. If the exercise is at all prolonged, however, the R.Q. falls off again.

It has been shown both for man and dog that prolonged and violent exercise can be taken in which the material consumed amounts to ten times the carbohydrate content of the entire body.† Whether under these circumstances fat or protein is consumed direct or whether these are first converted into carbohydrate is uncertain, but it would seem most probable that it is the latter.‡

With very intense exercise the R.Q. of the excess metabolism may be very high (up to 1·7), a result as yet unexplained.§

THE 'MECHANICAL EFFICIENCY' OF HUMAN EXERTION. It is possible to calculate the amount of mechanical energy liberated (theoretical maximal work) by men carrying out various exercises under normal conditions, and then by determining their oxygen utilisation during the work and recovery, to compute the mechanical efficiency of the performance. This is under favourable conditions about 20 per cent.|| Many approximations have to be made and sources of error allowed for, but it is surprising with what

* FURUSAWA. *Proc Roy Soc.*, 1925, **98B**, 65.
† DILL, JONES and EDWARDS. *le travail humain*, 1934, **2**, 1.
‡ GEMMILL. *Bull. Johns Hopk. Hosp.*, 1940, **66**, 71.
§ BEST, FURUSAWA and RIDOUT. *Proc. Roy. Soc.* 1929, **104B**, 119. SOLANDT and RIDOUT. *Proc. Roy. Soc.*, 1933, **113B**, 327.
|| DICKINSON. *J. Physiol*, 1929, **67**, 242.

constancy an experienced athlete can perform. In running, which has been chiefly studied, the allowances to be made include those for starting up and stopping and for onset of fatigue. In running, little actual mechanical work is done, the energy set free being used for imparting velocity to the body, a good deal of it being lost in overcoming viscous or other resistance in the muscles and other structures. The speed reached depends on the ratio of propelling force to resistance, chiefly that of his own muscles, as viscosity, and that represented by starting and stopping the movements. The resistance of the air is normally small, and may be allowed for. The superiority of first-class athletes over others of equal muscular strength appears to be due to the smaller 'viscosity' of their muscular apparatus, using this term in its widest sense to mean that in them less force is wasted in overcoming the resistances represented by putting their limbs in motion.

In maximal performances, such as those involved in athletic records, the man is exhausted when his oxygen debt reaches, say, 15 l., while his

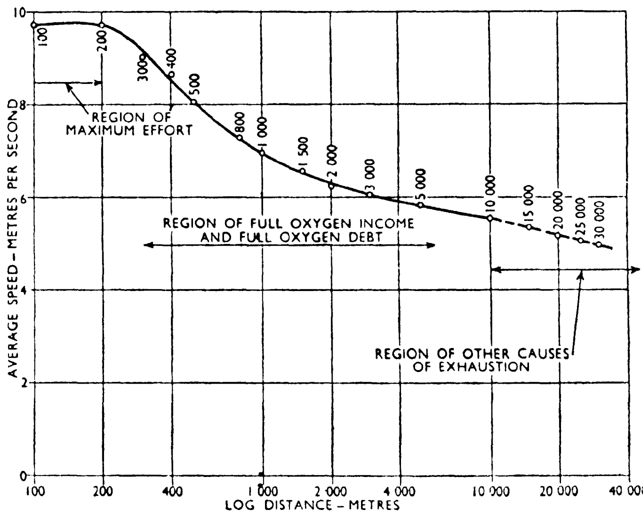


FIG. 75 Athletic records. Average speed plotted against log distance (A V HILL)

maximal oxygen intake is usually about 4 l./min. Lash, who created a world's record for a 2-mile run, used 5·35 l./min. when running at 21·6 km./hr.: in runs at 18·7 km./hr. for 5 min. he reached 5·1 l./min and at the finish had only about 48 mg /100 of blood lactate.* Hence a man can do work at a greater rate the shorter the total time for exhaustion, so that there is a close relation in athletic records between distance covered by a runner at exhaustion and the speed at which he covers it, as shown by Fig. 75.

During really maximal exertion lactic acid is produced at about 3 to 4 g./sec., and as a maximal tolerable amount is about 90 grammes, it is calculated that the severest exertions can only last for about 30 sec. This agrees with the practical experience of athletes.

OTHER CHEMICAL CHANGES IN CONTRACTION

Various other chemical changes have been found to occur when muscles are made to contract: the most important of these are changes in adenyl pyrophosphate and in

* v. SCIENCE, 1937 **85** 409.

creatine phosphoric acid. All the reactions which occur are closely inter-dependent, but are too complex to be fully described here.

The earliest change in contraction occurs in the adenyl pyrophosphate of the muscle, which is broken down into adenylic acid (= phosphoric acid — γ-ribose — adenine) and phosphoric acid. It is, however, very quickly resynthesised in fresh muscle. Later, when the muscle is fatigued, some of the adenylic acid is deaminated with formation of the mononucleotide inosinic acid (= phosphoric acid — γ-ribose — hypoxanthine) and ammonia.

$$\text{Adenyl pyrophosphate} \begin{cases} \nearrow \text{phosphoric acid} \\ \searrow \text{adenylic acid} \longrightarrow \text{Inosinic acid} + \text{ammonia} \end{cases}$$

At a later stage the unstable creatine phosphoric acid rapidly breaks down into creatine and phosphoric acid. Almost immediately after the contraction, even in absence of oxygen, part of the creatine phosphoric acid is resynthesised, and in presence of oxygen it is all rapidly reformed.*

CONTRACTION WITHOUT LACTIC ACID FORMATION. The correlation between muscular contraction and lactic acid formation led, very naturally, to the view that the initial reaction was the conversion of glycogen into lactic acid, with the corollary that the presence of the lactic acid in the muscle in some way caused it to contract, or, if in sufficient amount, led to rigor. It is now evident that we must go back further, and that the lactic acid formation is itself a sequel to earlier changes. This, while adding further detail, in no way invalidates what had been discovered concerning the lactic acid cycle.

The new viewpoint arose in consequence of the discovery by Lundsgaard in 1930 † that it is possible to get contraction without lactic acid formation. This was in muscles poisoned by small amounts of monoiodoacetic acid. If a frog's muscle be soaked for 30 minutes in oxygenated Ringer's solution containing 1 : 20000 of sodium iodoacetate it can still respond by contraction to stimulation. When it has given 100 to 150 contractions, however, it is fatigued. Whereas in unpoisoned muscle this would lead to the formation of about 0·05 per cent. lactic acid, in the poisoned muscle there is no increase of lactic acid at all. When the poisoned muscle has once been fatigued it fails to recover when allowed to rest in oxygen, as a normal muscle would do, and soon passes into rigor; and in this, instead of the normal high concentration of lactic acid, there is found to be no increase over the resting value. The glycogen, however, disappears as in the normal muscle.

From these facts it is evident that lactic acid formation is not an essential concomitant of contraction; yet when lactic acid formation is inhibited the muscle is only capable of a limited amount of contraction.

When the creatine phosphate of the muscle is studied after poisoning, it is found that it is broken down much in the normal manner, but, unlike the unpoisoned muscle, there is no resynthesis of creatine phosphate when the muscle is allowed to rest in absence of oxygen, though there is some restoration in oxygen: moreover, the phosphate set free is converted to a great extent into hexose monophosphate and diphosphate.

It is now believed ‡ that it is the breakdown of adenyl pyrophosphate or some still earlier, and as yet unknown, change which supplies the energy of the contraction. As will be explained later, the energy for rebuilding the adenyl pyrophosphate is derived from breakdown of creatine phosphate, that for resynthesis of creatine phosphate by breakdown of glycogen to lactic acid, and that for reformation of glycogen by the oxidation of part of

* EGGLETON and EGGLETON. *J. Physiol.*, 1928, **65**, 15

† LUNDSGAARD. *Bioch. Ztschr.*, 1930, **127**, 5, 162.

‡ On the basis of experiments with muscle extracts.

the lactic acid. It would seem that monoiodoacetic acid checks the formation of lactic acid by interfering with the breakdown of the triose phosphate, and also with further stages on the path of lactic acid formation. Fluorides poison the conversion of phosphoglyceric acid to phosphopyruvic acid.

We may now schematically represent the main chain of reactions which attend muscular contraction and recovery :—

$$\text{(1) Adenyl pyrophosphate} \underset{(b)}{\overset{(a)}{\rightleftarrows}} \text{Adenylic acid} + 2H_3PO_4.$$

$$\text{(2) Creatine phosphate} \underset{(d)}{\overset{(c)}{\rightleftarrows}} \text{Creatine} + H_3PO_4.$$

$$\text{(3) Glycogen} \underset{(f)}{\overset{(e)}{\rightleftarrows}} \text{Lactic acid.}$$

$$\text{(4) Lactic acid} + 3O_2 \xrightarrow[(g)]{} 3CO_2 + 3H_2O.$$

Of these reactions, (*a*)—(*c*) are anaerobic, (*d*) is partly anaerobic, but only complete in presence of oxygen, (*e*) is anaerobic, and (*f*) and (*g*) are aerobic.

PROBABLE SEQUENCE OF REACTIONS. Associated with the contraction of muscle are a remarkable series of interlocked reactions, which are the most fully studied changes in intermediary metabolism up to the present time. Such an advanced topic in biochemistry can only be dealt with in brief outline here, but it is important because there is reason to believe that the intermediary metabolism of carbohydrate in other tissues proceeds by similar stages.

Most of the changes studied are anaerobic ones, and many of the reactions concerned have been reproduced in cell-free extracts or solutions. Most of the changes too, are recovery changes, *i.e.* they occur *after* the actual contraction of the muscle, and serve to prepare it for the next contraction. Several of the reactions are coupled ones, *i.e.* two separate reactions run simultaneously, and the occurrence of one of them depends on the possibility for the concurrence of the other. This is often because one of them is endothermic and the other exothermic ; the endothermic reaction cannot occur unless the other is proceeding simultaneously and thus able to provide the necessary energy required for the first.

Of the substances involved some are individual compounds, and some are teams, or systems. Let us briefly review them. The chief are :

(1) *Carbohydrates and immediate derivatives, e.g.* glycogen, and phosphorylated compounds, such as phosphoric acid compounds of hexoses, trioses, pyruvic acid, and glycero-phosphoric and phospho-glyceric acids (formulæ on pp. 31, 36 and 142). These are all stages in the formation of lactic acid from glycogen.

(2) Various *enzymes* which bring about the reactions, *e.g.* the myozymase which converts glycogen to lactic acid. It is a mixture of several enzymes, mainly dehydrogenases.

(3) *Adenyl pyrophosphate* (formula on p. 142). This, in a coupled reaction, supplies the phosphoric acid for the first phosphorylation of glycogen.

(4) *Creatine Phosphate* (formula, p. 142). This restores H_3PO_4 to some of the adenylic acid reforming adenyl pyrophosphate.

(5) The *coenzyme system* for myozymase. This is a team consisting of—

(*a*) Adenyl pyrophosphate.
(*b*) Mg ions.
(*c*) Cozymase.

Cozymase is widely distributed in animals and plants, and is a compound of adenine, nicotinamide, pentose and phosphoric acid (formula on p. 105). It often acts in association with a dehydrogenase, becoming reduced, *i.e.* it acts as a hydrogen acceptor. Its action is then coupled with that of—

(6) *The Yellow Enzyme* (*i.e.*, lactoflavine phosphate, or its derivative: formula, p. 105). This takes up the hydrogen from the cozymase-dehydrogenase complex, restoring the cozymase to the oxidised form, and being itself reduced (v. p. 105).

(7) *Oxidised Cytochrome-c.* This restores the yellow enzyme complex to the oxidised state, and is itself reduced.

(8) Free oxygen which reoxidises cytochrome.

(9) Co-enzyme of pyruvate carboxylase.

The chemical changes concerned directly in lactic acid formation are shown in outline in the following scheme :—

Glycogen + APP $\longrightarrow$ fructose 1 : 6 diphosphate + A A.

$\downarrow$

$\rightarrow$ 2 Pyruvate + 2 Triose phosphate

$\downarrow$ (*blocked by I.A A.*)

2 lactate + 2, glyceric acid 3-phosphate

$\downarrow$

2, glyceric acid 2-phosphate

$\downarrow$ (*blocked by NaF*)

2 *enol*-phospho-pyruvic acid + A A.

2 Pyruvate + A.P.P.

A.P.P. = adenyl pyrophosphate ; A.A. = adenylic acid ; I A A iodoacetic acid. Triose phosphate = Glyceraldehyde 3-phosphate + Dihydroxyacetone phosphate.

In very brief summary, some of the chief reactions are :—

(A) *Phosphorylation of glycogen* with formation of hexose diphosphate.

Glycogen + $H_3PO_4 \longrightarrow$ hexose monophosphate . . . . . (1)

Hexose monophosphate + adenyl pyrophosphate + $H_2O \longrightarrow$ Hexose diphosphate + adenylic acid + H_3PO_4 . . . . (2)

By these two reactions the H_3PO_4 is kept available.

(B) *Formation of triose derivatives.*

Hexose diphosphate $\longrightarrow$ 2 Triose phosphates . . . . . . (3)

2 Triose phosphates + $H_2O \longrightarrow$ Phosphoglyceric and glycero-phosphoric acids. . . . . (4)

(C) *Formation of lactic acid.*

Phosphoglyceric acid $\longrightarrow$ Phosphopyruvic acid . . . . . . (5)

Phosphopyruvic acid + triose phosphate $\longrightarrow$ lactic acid + Phosphoglyceric acid . . . . (6)

(D) *Reinstatement of adenyl pyrophosphate* from adenylic acid formed in (2).

2 creatine phosphate + adenylic acid $\longrightarrow$ creatine + adenyl pyrophosphate . (7)

2 phosphopyruvic acid + adenylic acid $\longrightarrow$ 2 pyruvic acid + adenyl pyrophosphate . . . . (8)

(E) *Re-instatement of creatine phosphate* from creatine formed in (7).

2 creatine + triose phosphate + $H_3PO_4 \longrightarrow$ 2-creatine phosphate + lactic acid . (9)

Creatine + phosphopyruvic acid $\longrightarrow$ creatine phosphate + pyruvic acid . (10)

It will be seen from these reactions that phosphate plays a very important part in the formation of lactic acid from glycogen, and that the phosphoric acid is passed on from one compound to another, and ultimately restored.

The *cozymase* probably plays a double *rôle*, in both cases by acting as a hydrogen acceptor. Firstly, some of the reactions above, *e.g.* reactions (4) and (6) are oxido-reductions—one molecule being oxidised and the other reduced. The cozymase here first takes up the hydrogen from the molecule to be oxidised, and then hands it over to the one being reduced. So, reaction 6 could be written in two stages :—

Triose phosphate + Co $\longrightarrow$ Phosphoglyceric acid + CoH_2 . . . (6a)

Pyruvic acid + $CoH_2 \longrightarrow$ Lactate + Co . . . . (6b)

METHYLGLYOXAL. This substance, pyruvic aldehyde, has been shown to be formed in certain fermentation processes, and has often been supposed to be an important intermediary in carbohydrate metabolism. Dakin and Dudley showed that an enzyme, which they called glyoxalase, is widely distributed in animal tissues, and converts methyl glyoxal into lactic acid.—

$$CH_3CO.CHO + H_2O = CH_3CHOH.COOH.$$

Whether methyl glyoxal is an actual intermediary remains still in doubt, because under certain circumstances muscle extracts can still convert glycogen, but not methyl glyoxal, into lactic acid. In the absence of glutathione, or if the SH group of glutathione is inactivated by iodoacetic acid, glyoxalase is inactive, so that glutathione is a co-enzyme for glyoxalase.

HYDROGEN-ION CHANGES DURING CONTRACTION. Muscle is provided with several types of buffer, by which its reaction is prevented from changing rapidly when lactic acid is set free during contraction.

First, we have the products formed when creatine phosphate breaks up: creatine has strong basic properties and at first prevents the muscle from becoming acid; in fact during the actual contraction, owing to their liberation, it may have a rather lower hydrogen-ion concentration than primarily. Then, in prolonged activity we have traces of ammonia liberated.

Next, we have the salts of proteins already referred to, which also possess considerable buffering power. In spite of these buffers the hydrogen ion concentration of the muscle does increase after prolonged activity, though not greatly.

FORMATION OF HISTAMINE. It has been claimed that during contraction, and also under the influence of curari, histamine is liberated from a pre-existing store in the muscle and may be detected in the venous blood from muscles.* The amount set free by curari is large and generally admitted, but the liberation of histamine by contraction is small, and some deny that any is formed.

FORMATION OF CARBON DIOXIDE. In the final oxidative reactions by which lactic or pyruvic acids are oxidised to CO_2 and water, it is probable that the cozymase acts in collaboration with the yellow enzyme and with tissue cytochrome, the hydrogen being passed under the influence of dehydrogenase enzymes, first from the oxidisable substance to cozymase, then to the yellow enzyme, then to oxidised cytochrome, and finally to free oxygen, which is reduced to water.

It seems that vitamin B_1 (aneurin) may play a direct part in one phase of carbohydrate metabolism. In combination with pyrophosphoric acid it acts as the co-enzyme to the enzyme which breaks down pyruvic acid.

Lactic acid dehydrogenase

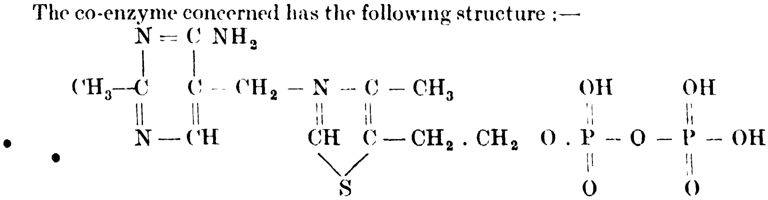
Lactic acid ——————→ Pyruvic acid

Oxygen

Carboxylase (with co-enzyme).

$CO_2 + H_2O$ ←—— Oxydation products.

The co-enzyme concerned has the following structure:—

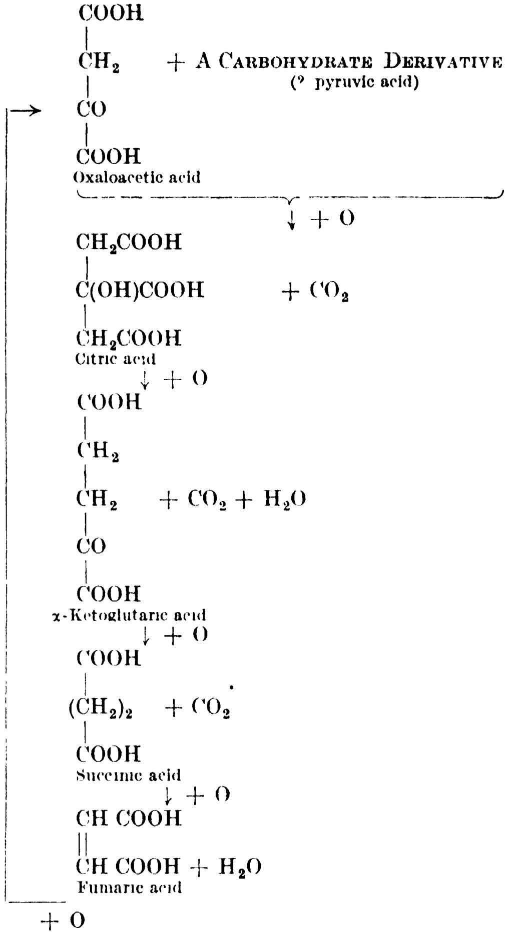


Co-enzyme of Pyruvate Carboxylase

(*Aneurin pyrophosphate*)

* ANREP ET AL. *J. Physiol.*, 1935, **85**, 409; 1939, **96**, 240.

It must be admitted that the final stages of aerobic metabolism in muscle, by which carbon dioxide is formed, are not yet clear. It seems that dicarboxylic acids with four carbon atoms play some part in it, and one such scheme * may be briefly outlined.



so that the net change is :—

$$\underset{\text{Pyruvic acid}}{CH_3COCOOH} + 5O = 3CO_2 + 2H_2O.$$

Another possibility, which helps to link up the metabolism of carbohydrate with that of fat and protein is indicated in the following reactions :—

(1) $\underset{\text{Pyruvic acid}}{CH_3CO\ COOH} + O \rightarrow \underset{\text{Acetic acid}}{CH_3COOH}$

(2) $\underset{\text{acid}}{\text{Pyruvic}} + \underset{\text{acid}}{\text{Acetic}} \rightarrow$ Acetopyruvic acid $CH_3CO\ CH_2CO\ COOH + H_2O$

(3) Acetopyruvic acid $\xrightarrow{O} \underset{\text{Acetoacetic acid}}{CH_3CO\ CH_2COOH} + CO_2$

$\searrow^{H_2O}$

$$\underset{\beta\text{-Hydroxybutyric acid}}{CH_3CH(OH)CH_2COOH} + CO_2.$$

It is suggested that this series of reactions may occur if for any reason the citric acid cycle breaks down. In that case, acetone bodies might be derived from carbohydrate as well as from fatty acids.

* Krebs, Salvin and Johnson. *Biochem. J.*, 1938, **32**, 113.

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THE HEAT PRODUCTION IN MUSCULAR CONTRACTION

Although chemical investigation has yielded valuable information as to the net chemical changes which occur, its results are limited inasmuch as it does not give reliable information as to the course of events occurring during the phases of contraction, relaxation and recovery. All we can do is to examine the summated results of a long series of contractions. A single twitch will give no detectable chemical change. On the other hand, every chemical change is associated with production or absorption of heat, and our

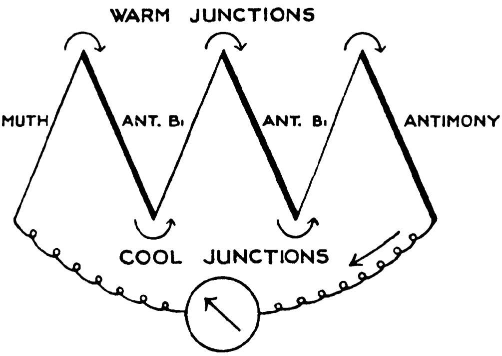

FIG. 76.

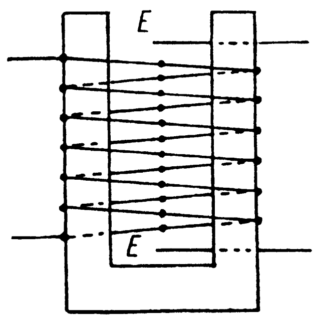

FIG. 77. Plan of Construction of Thermopile. (HARTREE)

methods for detecting small differences of temperature are so exact that we are able to measure not only the heat production during a single twitch but also the time-course of its evolution, and are thus in a position to assign the different chemical events to the appropriate phase of muscular activity.

The experience of everyday life teaches us that muscular exercise is associated with increased production of heat. In large animals the production of heat in muscular contraction can be easily shown by inserting the bulb of a thermometer between the thigh muscles, and stimulating the spinal cord. This observation is confirmed when we investigate the contraction of an isolated muscle outside the body. If a frog's muscle at 20° C. is tetanised, its temperature rises about 0·03° C. per second of stimulation, and for each single twitch from 0·001° to 0·005° C.

Such small changes in temperature as 0·001° can be estimated by thermoelectric junctions. The principle employed depends on the fact that, when two or more junctions of a circuit made of two metals are at different temperatures, a current of electricity flows through the circuit, and can be measured by means of a galvanometer. Thus in the circuit (Fig. 76) composed of two metals, antimony and bismuth, if the upper junctions be warmed there will be a current flowing in the direction of the arrows, and this current will within limits be proportional to the difference of temperature between the warm and cool junctions. In such a series of junctions, alternately warmed and cooled, the currents are added together; this is the thermopile.

To measure the rise of temperature in a muscle during its contraction, the thermopile is made in the form of a grid. It consists of forty or more turns made with alternate segments of fine iron and constantan wire soldered together with silver solder and wound between the arms of a U-shaped insulating frame (Fig. 77) so that two sets of junctions lie half-way between the arms of the U and the other two sets on the outside edges of these arms. A slip of mica is inserted between the front and back parts of the spiral, and the wire insulated throughout with a coating of shellac varnish. A pair of muscles (*e.g.* sartorii) is used, and is arranged so as to straddle the grid, each muscle lying close against the middle ("warm") junctions, while the outer junctions are maintained a a constant temperature. For this purpose the arrangement shown in Fig. 78 may be used. Through the rubber bung, R, of a large test tube pass three glass tubes, G. Between these the thermopile is fastened. The leads from the thermopile to the galvanometer pass through two holes in one of the glass tubes. The muscle is stimulated by the electrodes, e_1 and e_2. The lower electrode e_2 is connected with the stimulating circuit by means of a copper wire leading up through the other glass tube. The upper electrode e_1 consists of an 8-shaped loop of platinum tied between the two muscles, and soldered to a fine steel rod, which makes electrical connection with the stimulating circuit, and mechanical connection with the lever which records the contractions of the pair of muscles. The whole apparatus is kept at as constant a temperature as possible. The leads from the thermopile are taken to a very sensitive galvanometer of low resistance.

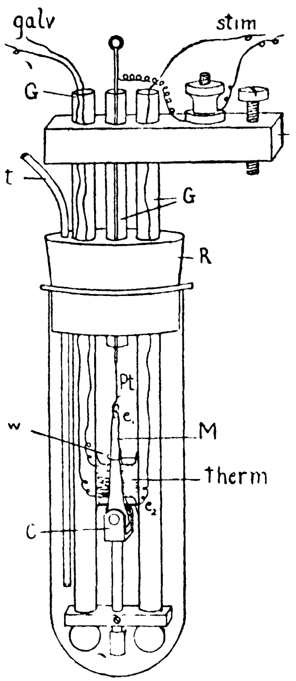

FIG. 78. Arrangement of Thermopile and Muscle for measuring Heat Production during Contraction. E is an ebonite block by which the whole apparatus is secured upright in the water bath ; *w* is a platinum wire guide for the muscle to insure good contact with the thermopile ; *t* is a fine glass tube through which Ringer's solution or oxygen can be introduced into the chamber ; C is an ebonite clamp to hold the muscle ; it is secured to the glass rods by means of an ebonite bar at the base and a silver-plated set screw. (W. O. FENN. *J. Physiol*, 1923, **58**, 175.)

The thermopile and galvanometer can be calibrated so that the changes of temperature in the muscle may be deduced directly from the galvanometer excursions. In order to convert these temperature changes into amounts of heat actually evolved, the muscle at the end of the experiment is killed, and the heating effect observed on passage of an interrupted current. Knowing the strength of the current and the resistance of the dead muscle, the exact amount of heat required to produce an observed deflection can be calculated.

Calorimetric Method. For the determination of the total heat production in a muscle or group of excited muscles over a considerable time we may use the microcalorimeters devised by A. V. Hill * and R. A. Peters.†

Two small Dewar flasks are used (Fig. 79). In each of these are suspended several frogs' muscles immersed in Ringer's fluid. They are arranged so that their nerves can be stimulated while in the flask. Thermo-junctions are immersed in the fluid in the two flasks. In this arrangement the heat loss or gain in the two vessels should be identical. Any difference of temperature between the two vessels as revealed by the galvanometer is therefore due to liberation of heat in the muscles which are being excited.

Valuable results have been obtained with the above-mentioned methods on the heat production during muscular contraction. By the calorimetric

* HILL. *J. Physiol.*, 1912, **43**, 261.
† PETERS. *J. Physiol.*, 1914, **47**, 243.

method it may be shown that in anaerobic muscular contractions the formation of 1 gramme of lactic acid is attended with the evolution of

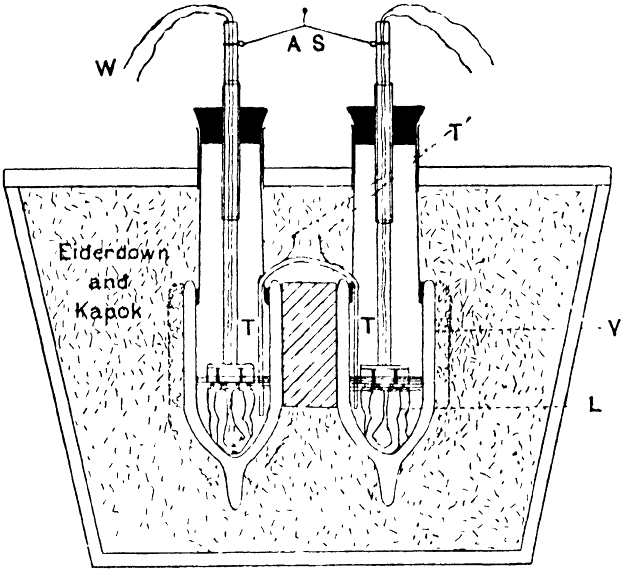

FIG. 79. Arrangement of two Dewar Flasks for studying the Production of Heat in Muscles over a considerable period of time (R. A. PETERS.)

about 370 gramme-calories.* The thermopile shows that this heat does not come off at once, but in two phases, there being an evolution of heat, "*initial heat*" immediately following on excitation, and a subsequent

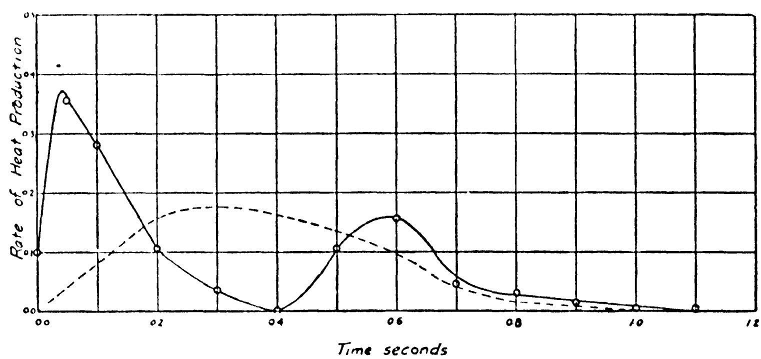

FIG. 80. Course of initial heat production (continuous line) and tension development (interrupted line) in a single twitch of a frog muscle. (HILL, after HARTREE.)

evolution of heat, "*anaerobic delayed heat,*" accompanying and following relaxation.

During recovery in oxygen there is, accompanying the disappearance of lactic acid, a further prolonged evolution of heat, "*delayed aerobic heat.*" It has been shown, by cooling frog muscles to 0° so that changes are slowed

* PETERS. *J. Physiol.*, 1914, **47**, 243.

down, that the time course of the oxidative delayed heat is the same as that of oxygen consumption.* As this is only a further 370 calories per gramme of lactic acid removed, only a part of the lactic acid can undergo oxidation to CO_2, since oxidation of 1 gramme of this substance involves the liberation of 3600 calories; and we must conclude, as suggested above, that the greater part of the lactic acid is reconverted, during recovery, into glycogen. As a matter of fact, though about one-fifth of the lactic acid undergoes complete oxidation, only part of the energy of this oxidation appears as heat, while the rest of it is absorbed in the endothermic reconversion of lactic acid into glycogen.

Isotonic Contractions. It has been shown by Fenn † that a muscle develops more heat on isotonic than on isometric contraction. This may be interpreted as meaning that the isotonic contraction does not merely utilise tension energy liberated at the commencement, as though the muscle were a stretched spring, but that it continues to develop further power as the shortening progresses. In accordance with this it has been shown that the relation between force exerted and rate of shortening is not as simple as the viscous elastic model (p. 137) would suggest, *i.e.* the decrease of force with increase in speed is not linear, as viscous change would demand, but exponential.‡

Isometric Contractions. The total initial heat formed in isometric contraction and relaxation is a measure of the total energy set free by the muscle in the act of its contraction, and, as Hill showed, the tension set up under isometric conditions bears a constant relationship to the heat production, the ratio Tl/H (where T = tension, l length of muscle, and H the heat produced) remaining constant at an average value of 8, under a variety of conditions. This ratio is a measure of the perfection with which the chemical potential energy is converted into mechanical potential energy, *i.e.* of the efficiency of the muscle.

Increase of initial extension causes a rise of the heat production, as well as of tension production, up to a certain maximum, beyond which there is a decline in both (*v.* Fig. 61).

Relation to Chemical Change. By analysis of the time relations of the process by the use of thermopiles, and correlation with the chemical changes as discussed above, the conclusion has been reached that the sequence of events as regards the heat production in muscular contraction and recovery is in outline as follows:

(1) Contraction. The first event is probably the breakdown of adenyl pyrophosphate into adenylic acid and phosphoric acid, with liberation of heat and energy:—

1 mol. adenyl pyrophosphate = 1 mol. adenylic acid + $2H_3PO_4$ (+ 25,000 cal.)

Then follows the breakdown of creatine phosphate:—

1 mol. creatine phosphate = 1 mol. creatine + H_3PO_4 (+ 11,000 cal.).

Part of the energy set free being absorbed in resynthesising adenyl pyrophosphate.

(2) Relaxation. Next, three reactions occur simultaneously:—

1 mol. hexose equivalent (glycogen) = 2 mol. lactic acid (+ 32,000 cal.).

2 mol. lactic acid neutralised by proteins, etc. (= + 16,000 cal.).

4 mol. creatine + 4 H_3PO_4 = 4 mol. creatine phosphate (− 44,000 cal.)

and, as will be seen, the net heat exchange is + 4,000 cal.

After relaxation has occurred there is formation of lactic acid and resynthesis of creatine phosphate. There is a further and much slower production of heat.§ In actual experiments on muscles, in absence of oxygen this, the delayed anaerobic heat, is much

* D. K. HILL. *J. Physiol.*, 1940, **98**, 207.

† FENN. *J. Physiol.*, 1924, **58**, 175, 373.

‡ FENN and MARSH. *J. Physiol.*, 1935, **85**, 277.

§ HARTREE and HILL. *J. Physiol.*, 1922, **56**, 367; 1923, **58**, 127. BLASCHKO. *J. Physiol*, 1930, **70**, 96.

less per g. lactic acid formed than we should calculate. The heat liberated represents the algebraic sum of at least four simultaneous processes:

(a) Lactic acid formation—evolution of heat	Total = +200
(b) Lactic acid neutralisation by protein-evolution of heat .	to 300 cal.
(c) Creatine phosphate resynthesis-absorption of heat . .	per g.
(d) Re-ionisation of protein-absorption of heat . . .	lactic acid.

When the muscle has been poisoned with iodoacetic acid, stage (1) occurs, but not stage (2), and as we have seen, there is no lactic acid formation. Hence it is concluded that the heat available from the formation of lactic acid does not all appear, since some of it is absorbed in the endothermic reactions (c) and (d) and some perhaps in other ways.

(3) In presence of oxygen the delayed heat is much increased (Recovery heat), though its rate of formation is still slow when compared with the initial heat.* This again is due to a balance of exothermic and endothermic processes:—

(a) Complete creatine phosphate resynthesis—(absorption of heat	total about 370 cals. per
(b) Oxidation of lactic acid (or carbohydrate)—(evolution) .	gramme L.A.
(c) Re-formation of glycogen (absorption)	removed.

The results of the thermal investigations thus enable us to supplement those obtained by chemical methods but final agreement has not yet been reached.

THE MECHANICAL EFFICIENCY OF CONTRACTION. When a muscle contracts it may perform mechanical work, and the energy for this work is derived from the chemical and physical changes associated with contraction and recovery. It is often necessary to consider the *mechanical efficiency* of contractions carried out under certain conditions, *i.e.* to know what fraction of the total energy set free in the contraction is converted into work. Thus if 10 per cent. of the energy set free were converted into work, the remaining 90 per cent. being liberated as heat, the mechanical efficiency would be 0·1 (or 10 per cent.).

The mechanical efficiency will vary, for a given muscle, with conditions, *e.g.*, with load lifted, rate of shortening, etc., but will of course never be anything like 100 per cent. In human muscles *in situ* it may be anything up to 20 per cent. and seems to be similar for frog muscle *in vitro*,† when the whole cycle of contraction and recovery is taken into account. For individual muscles speed of contraction largely influences the efficiency, *e.g.*, for arm flexion to full extent the optional time is 0·5 sec., and for a leg flexion about 1 sec. With an isometric contraction, or an isotonic one without any load, the efficiency is, of course, nil.

Mechanical efficiency is measured by finding the work done in the usual way (weight $\times$ height) and finding what fraction this is of the energy expended as found by measuring either total heat production, or total oxygen usage. (Knowing that 1 g. cm. = 23·4 micro cal. = $4{\cdot}7 \times 10^{-6}$ c.c. oxygen usage).

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ELECTRICAL CHANGES IN MUSCLE

About the middle of the eighteenth century there arose the suggestion that excitation was essentially an electrical phenomenon, and it was supposed by some that tissues could develop electrical potential differences.‡ The fact that electrical currents can be obtained from living tissues was proved, probably by Galvani in 1794 § by his demonstration of "contraction without metals," as described below. It is easy with present-day apparatus to show that all living tissues when active exhibit differences of electrica potential, and these phenomena are of considerable practical and theoretical importance. Much of the description given here for muscle applies equally well to other tissues.

POLARIZATION. If a current from a battery be passed between two plates of platinum immersed in acidulated water or salt solution, *electrolysis* of the water takes

* HARTREE. *J. Physiol.*, 1932, **75**, 273; **77**, 104.
† HILL. *Proc. Roy. Soc.*, 1939, **127B**, 434; **128B**, 263.
‡ HOFF. *Annals of Science*, 1936, I. 157.
§ The publication of 1794, the famous "*Dell' arco conduttore*," was anonymous. v. FULTON and CUSHING. *Annals of Science*, 1936, I. 239.

place, bubbles of oxygen appearing on the positive plate (anode), and bubbles of hydrogen on the negative plate (cathode). If now we remove the battery, and connect the two plates (electrodes) by wires with a galvanometer, a current passes through the galvanometer and water in the reverse direction to the previous battery current. This current is called the polarization current, and is due to the electrolysis of the water that has taken place The vessel in which the electrodes are immersed has in fact become a storage cell, the platinum covered with oxygen bubbles being the positive *element,* and that covered with hydrogen bubbles the negative *element.* Similar polarization takes place when we pass currents through the tissues of the body by means of metallic electrodes.

We have already learnt in Chapter VIII that the electrical phenomena of living tissues are due to the existence of polarized membranes at the surfaces of the cells ; *i.e.* of membranes having a positive charge on one surface, usually the outer one, and a negative charge on the other surface. It is therefore essential in studying such phenomena to avoid other forms of polarization, which would mask or modify them.

Hence, before we can study accurately the electrical changes that occur normally in living tissues, it is necessary to have some form of electrodes in which this polarization will not occur. With one exception, polarization always results when a current passes from an ordinary metallic conductor to a saline solution, but not when it passes from one saline solution to another. This exception is when the metal dips into a solution of its own ions, and this fact is taken advantage of in the construction of *non-polarizable electrodes.* In these a metal dips into a solution of its salt ; any tendency for polarization is at once

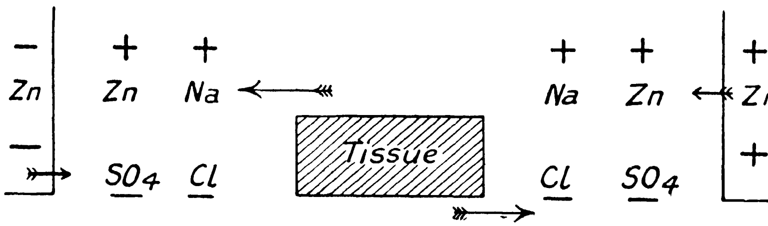

FIG. 81.

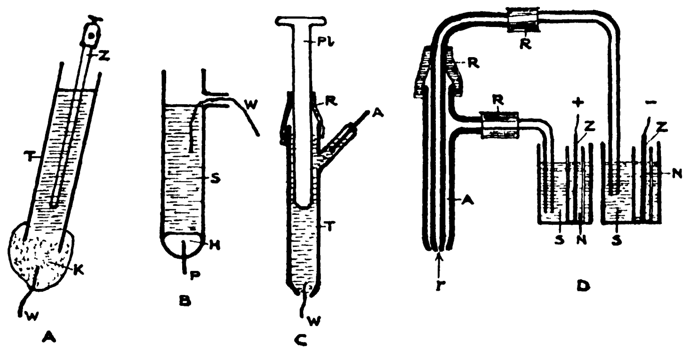

FIG. 82. Types of Non-polarizable Electrodes.

(A) Noyons' type of non-polarizable electrode. Z = amalgamated zinc rod imbedded in 10 per cent. gelatine containing $ZnSO_4$ and contained in the glass tube, T. K = plug of kaolin made with isotonic NaCl solution. W = thread wick soaked in isotonic NaCl leading to tissue.

(B) Ostwald type of N.P.E. P = platinum wire making contact with mercury H. S = isotonic NaCl solution containing solid calomel. W = saline wick lead to tissue.

(C) Lapicque's AgCl electrode. A = silver electrode coated with AgCl., Pl = plunger fitting rubber sleeve R, for filling electrode with NaCl solution. W = saline wick lead.

(D) Pratt's pore electrode. r = the fine pore which is the stimulating point. The tube is filled with saline solution and connected with the saline solution in the vessel S, in which is a porous N.P.E. cell filled with $ZnSO_4$ solution and having a Zn rod immersed in it. The outer tube, A, is the other electrode and is similarly connected with the other N.P.E. cell. R = rubber connections.

counteracted at the cathode by deposition of the metal on to the electrode from the solution, or at the anode by solution of the metal of the electrode to neutralise the negative charges carried thence by anions (Fig. 81). Some types of these electrodes are shown diagrammatically in Fig. 82.

In such electrodes the conduction of the current through the nerve or muscle to the metallic part of the circuit may be represented as shown in Fig. 81.

If a muscle such as the sartorius be removed from the body, and two non-polarizable electrodes connected with a delicate galvanometer be applied to two points of its surface, there will be a deflection of the galvanometer, showing the presence of a current which flows in the external circuit from uninjured to injured surfaces. This current of resting muscle is due to the excitatory effects of injury. The less the preparation is injured, the smaller is the current to be obtained from it. Hermann described the fact of the existence of currents of rest thus : " In partially injured muscles every point of the injured part is negative towards the points of the uninjured surface." These phenomena are explained in Chapter VIII, Figs. 30—32. When the injured fibres are quite dead, this current of rest or ' demarcation current ' disappears. The current is due to the electrical differences at the junction of living and dying (not *dead*) tissue. If the sartorius of the frog be cut out and immersed for twenty-four hours in Ringer's solution, all the injured fibres die, and the uninjured fibres are then found to be iso-electric and therefore currentless.

If one end of the muscle be now injured by cutting or burning, a galvanometer will show the presence of the current of injury, or demarcation current between injured and uninjured tissue. The existence of this current may be demonstrated as in Galvani's classical experiment, without using a galvanometer. If the nerve of a sensitive muscle-nerve preparation *a* (Fig. 83) be allowed to fall on an excised muscle *b*, so that one point of the nerve is in contact with the cut end and one with the surface of the second muscle *b*, the muscle *a* will contract each time the nerve touches *b* so as to complete the circuit. If the nerve of *b* is tetanised, *a* as well as *b* enters into a tetanic contraction, for reasons that will be clear later.

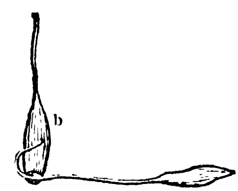

FIG. 83. Galvani's "Contraction without metals."

Although resting uninjured muscle shows no current, a definite electrical change occurs in it when it contracts. As we saw in Chapter VIII these changes are due to depolarization of the membranes at the active places. To show this change, we may lead off two points, one on the cut end and one on the surface of the muscle of a muscle-nerve preparation, to a galvanometer. We shall first obtain a deflection of the mirror due to the "*current of rest*" (or "*injury current*," or "*demarcation current*"), the cut surface being negative to the resting uninjured surface. If now the nerve be stimulated with an interrupted current so as to throw the muscle into a tetanus, the galvanometer mirror swings back towards the zero, showing that the current which was present before is diminished, *i.e.*, the uninjured surface on excitation also becomes negative, so that little or no current now flows from it to the injured part. When the excitation of the nerve is discontinued, the galvanometer indicates once more the original current of rest. This diminution of the current of rest during activity is spoken of as the ' negative variation.'

In order to obtain a graphic record showing the time-relations of the electrical changes accompanying a single muscle twitch, it is necessary to employ some instrument which

can react much more rapidly than the ordinary galvanometer. For this purpose we may employ either the capillary electrometer, the string galvanometer or a suitable oscillograph.

The Capillary Electrometer is an instrument for recording difference of potential. Its structure is very simple. It consists of a glass tube drawn out to a fine capillary point. This tube, with the capillary, is filled with mercury. The point dips into a wide tube containing dilute sulphuric acid, at the bottom of which is a little mercury. Two platinum wires fused into the glass and dipping into the mercury serve as terminals. When the instrument is used, the meniscus of the mercury in the capillary at its junction with the acid is observed under the microscope (Fig. 84), or a magnified image of it is thrown on a screen with the aid of an electric

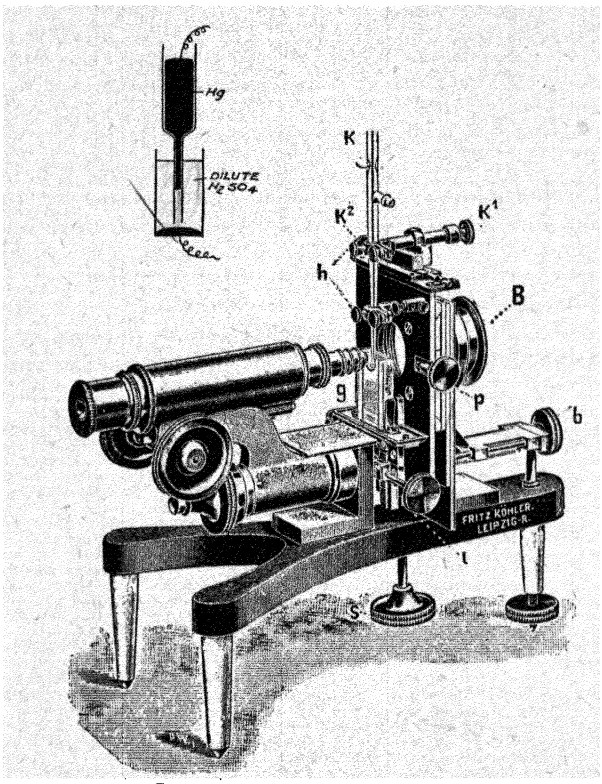

FIG. 84.—Capillary Electrometer, with microscope, according to F. B. Hofmann. The capillary tube, shown in diagram above, is on the path of a beam of light, which enters at B. The magnified image of the meniscus in the capillary is projected by the microscope on to a viewing screen or moving photographic surface.

arc. If now the capillary and acid be connected with two points, it will be observed that any difference in the potential of these two points causes a movement of the meniscus in the direction in which the current is flowing, and the extent of the excursion varies with the difference of potential. The excursions lend themselves well to photography, so that we may obtain a graphic record of every electrical variation, and thus determine its extent and its time relations. Somewhat elaborate corrections of the records are necessary however.*

It must be remembered that this instrument is an electrometer (measurer of difference of potential), and not a galvanometer (current measurer) so that no current passes the surface. Hence the use of non-polarisable electrodes is not so essential as when we make use of the galvanometer.

* LUCAS. *J. Physiol.*, 1912, **44**, 225.

In the moving-coil galvanometer the current is sent through a coil of fine wire hung between the poles of a powerful magnet. The same princ ple in simpler form is made use of in tne *String Galvanometer of Einthoven* (Fig. 85). In this a very delicate thread, of silvered quartz or of platinum, is stretched between the poles of a strong magnet. The poles of the magnet are pierced by holes so that the thread may be illumined by an electric light from one side, and from the other a magnified image of the thread may be thrown upon a screen. Whenever a current

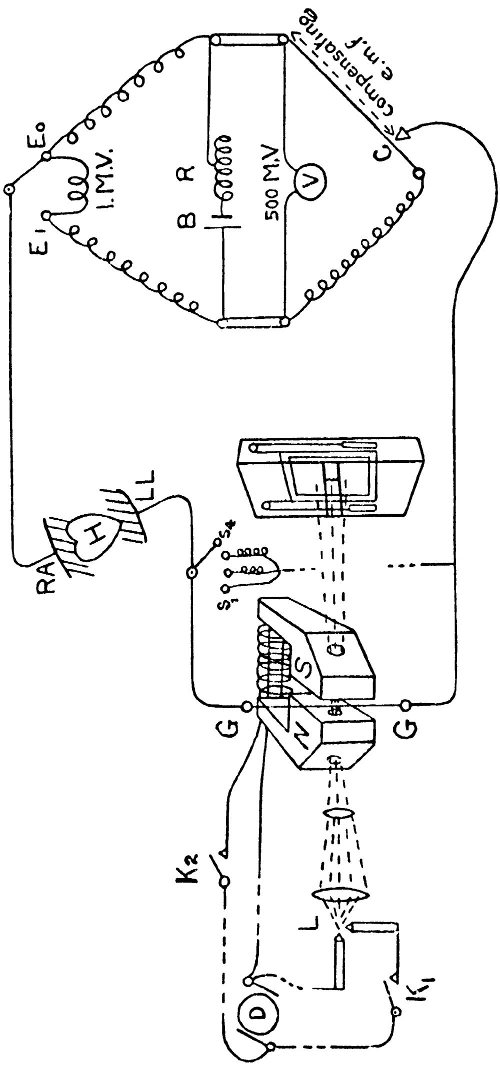

FIG. 85. Diagram of the connections of the String Galvanometer, as arranged for electrocardiography. D = source of current; L = arc-light and lens system; N.S. = the magnet; G.G. = the thread; S_1 to S_4 = shunt for varying flow through galvanometer circuit. The light falls on the camera with vertically moving plate and horizontal slit. On the right the compensating apparatus. Leading off from right arm (R.A.) and left leg (L.L.) gives record of potential changes in heart (H). (D. T. HARRIS.)

passes through the thread it moves laterally, and the lateral movement may be photographed on a moving photographic plate. Owing to the minute dimensions of the thread the instrument is one of extreme delicacy. It will detect very minute currents and will respond reasonably accurately to very rapid changes in potential. The records of rapid fluctuations are more nearly correct than those obtained by the capillary electrometer, but such errors as are present are less amenable to correction.

For very accurate work neither instrument is entirely satisfactory, and it is better to employ a rapidly moving *oscillograph*, such as the cathode ray oscillograph, or

the mechanical one devised by Matthews.* This resembles a loud speaker, the plate being replaced by an iron tongue to which a small mirror is attached. The current to be recorded is amplified appropriately by means of valves. A beam of light is reflected from the mirror, the movements of which can be photographically recorded (Fig. 86)

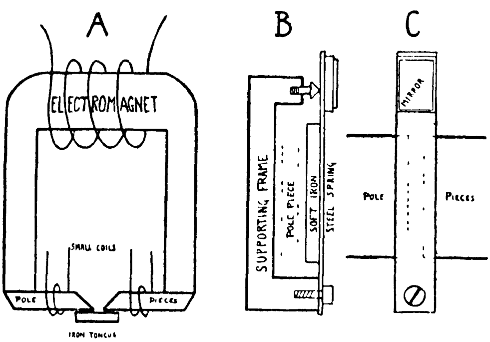

FIG. 86 Diagram of Matthews' Oscillograph B and C show section and front view of tongue and mirror. (MATTHEWS)

DIPHASIC AND MONOPHASIC POTENTIAL WAVES. If an uninjured regular muscle (Fig. 87) such as the sartorius be kept in moist air and stimulated with a single induction shock at one end, x, or indirectly, and two point s, a and b, be led off to a suitable instrument, *e.g.* a string galvanometer, each stimulus applied at x gives rise to a *diphasic* electrical variation. Our study of the mechanical events in muscle has shown that, when the muscle is stimulated at x, a contraction wave commences which travels down the muscle through a to b. The electrical investigation of the muscle shows that excitation of x arouses an electrical change which also passes down the muscle at the same rate as the mechanical change, which it slightly precedes. On leading off from a and b there is a latent period between the stimulus and the first change, representing the time taken for the change to travel from x to a. When the change reaches a, this becomes the seat of a depolarization, the membranes previously positive becoming discharged. Hence the current would pass in the outer circuit from b, which is still positive, to a. We may say therefore that a is negative to b. A fraction of a second later the excitatory change has passed on to b and has died away at a. Now b is negative to a, and the current therefore passes in the opposite direction. Between a and b, therefore, there

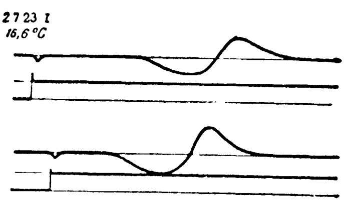

FIG 87 Diphasic action potentials in gastrocnemius muscle of frog, recorded by an oscillograph Below each tracing is the signal line showing stimulation of the sciatic nerve The upper tracing shows stimulation of central end of the nerve, the lower one of the peripheral end. The time-line below indicates 0 01 sec. (ROSENBERG. *Pflüger's Arch*, 1929, **223**, 120)

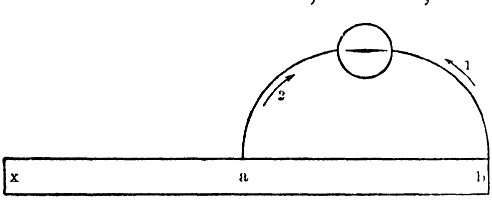

FIG. 88. Diagram showing Diphasic Variation of uninjured Muscle.

is a diphasic current, the first phase representing negativity of a to b, and the second phase negativity of b to a. *A diphasic change is thus also a sign of a propagated change,* Every excitation of a normal muscle gives rise to a diphasic variation of such a direction that the point stimulated first becomes negative to all other points of the muscle, and this 'negativity,' to use a loose but convenient expression, passes as a wave down the muscle, preceding the wave of contraction and travelling at the same rate (Fig. 88).

* MATTHEWS. *J. Physiol.*, 1928, **65**, 225.

If one leading-off point be injured, *e.g.* at *b*, that point becomes permanently negative (injury current), and the change accompanying excitation is absent at that point. A single stimulus applied at *x* will in this case give only a *monophasic* variation in which *a* is relatively negative to *b* (negative variation of injury current).

When we study the time relation of the electrical variation ensuing on a single stimulus, we find that the electrical change under the electrodes begins at the moment that the stimulus is applied. It takes for frog muscle about 0·0025 second to attain its culminating point. At this point the mechanical change or contraction of the muscle begins. These time relations vary with the temperature of the muscle. We have already seen that the effect of lowering the temperature is to increase that latent period of the contraction. In the same way it slows the rise of the electrical change and the rate of propagation of the wave of electrical change. We are therefore justified in regarding the electrical change as an index to the ionic changes evoked in the muscle as the direct result of the stimulus. The material change in form of each contracting unit is secondary to these changes. As the result of stimulation, depolarization is aroused at the point of excitation, and travels thence along the muscle fibre at a rate of about 3 metres per second, *i.e.* the same rate as that of the following wave of mechanical change and, like this, varying with the temperature.

Under no conditions, however, may an excitatory condition be propagated without the presence of a contraction, though often this may be so small as to be difficult of detection. When a muscle is so treated as to diminish its mechanical response, *e.g.* by soaking in water, the electrical response is also reduced, and the two finally vanish together when the muscle passes into "water rigor."

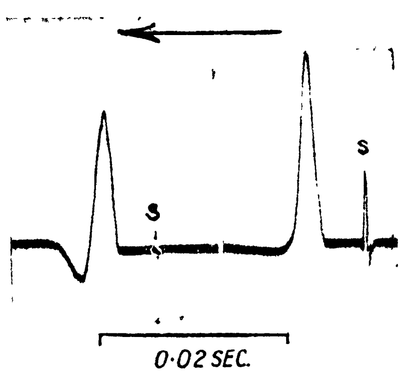

FIG. 89. Diphasic (left) and monophasic action (right) action potentials in non-medullated nerve of Maia, recorded by cathode ray oscillograph. Point of stimulation shown by S. Read from right to left. Time indication = 0·02 sec. (Tracing by Dr. J. Yule Bogue.)

The phenomena of diphasic and monophasic action potentials are shown with even greater clarity in nerve than in muscle. If two uninjured points on a nerve trunk are led off to an oscillograph, a diphasic potential wave can be recorded at the passage of each excitation applied to the nerve, and indicates the transmission along the nerve of a nerve impulse. If one of the points led off has been injured, *e.g.* by burning with a hot wire, then each nerve impulse passing the other, uninjured, point occasions a monophasic action potential (Fig. 89). For further details of nerve action potentials, see Chapter 89.

Somewhat similar tracings can be obtained from almost any tissue when excited.

OTHER PHYSICAL CHANGES ASSOCIATED WITH CONTRACTION

Various other physical changes are associated with contraction; thus there are changes in double refraction (diminished), volume (increased), translucency (diminished), and impedance (increased).* Although these changes have not been very extensively studied, the close correspondence between their time relations and the time relations of the tension curves and heat production indicate that the processes are closely related.

* DUBUISSON. *J. Physiol.*, 1937, **89**, 132.

Generally speaking, the curves for all these changes show two phases, one during the contraction and the other during relaxation. The former is thought to be related to changes in adenyl pyrophosphate, and the latter to creatine phosphate and lactic acid changes. In muscles poisoned with iodoacetic acid, or under anaerobic conditions, the time relations of all five changes are also very similar.

THE INTIMATE NATURE OF MUSCULAR CONTRACTION

The means by which the chemical energy of the contracting muscle is converted into the mechanical energy of the contractile process is quite unknown. Accordingly, theories are numerous in comparison with relevant facts.

We know now that there can be no question of a conversion of heat energy into work, because the heat is generated after the work is done, and also because the necessary high temperature could not be reached without killing the muscle.

Experiments on the metabolism of the body as a whole show that the performance of work involves an increase of the oxidative processes of the body with a corresponding evolution of energy, of which four-fifths will appear as heat while at most one-fifth may be transformed into mechanical work.*

The mechanism in the fibre must be one which will provide for a more or less direct transformation of chemical energy into mechanical energy. It is probable that contraction is essentially a physical process, as say, the uncoiling of a spring is a physical process and that all chemical changes, including that of adenyl pyrophosphate breakdown, are subsequent and serve to restore the energy for the physical process and so, as it were, rewind the spring. Of the nature of these consequential chemical changes we have now considerable information, but the essential nature of the physical changes is still a matter for speculation. Some theories regard the chemical changes as the fundamental ones and the physical changes as coming later. In the muscle fibre we have heterogeneous systems consisting largely of colloids bathed in solutions, and most of the energies may be reduced to one of three kinds, osmotic energy, electrical energy and surface energy. The contractile machine might therefore be one which employs one or more of these forms of energy. We might, for instance, consider the question from the viewpoint of histological structure. When the muscle is at rest the contents of the sarcomere will be in equilibrium with the surrounding sarcoplasm. We might imagine the excitatory process to consist in a sudden chemical change occurring in the contents of the sarcomere. The production of a number of new molecules (*e.g.* by breakdown of phosphagen) would raise the osmotic pressure and occasion a rapid flow of water from the sarcoplasm. As a result the pressure in the sarcomere would rise and cause a bulging of its lateral wall and a shortening of the whole element.† The subsequent phase of relaxation may be due either to a secondary change, *e.g.* neutralisation, leading to the formation of a substance to which the walls of the prism are freely permeable, or to the gradual leak of the primary products of oxidation or disintegration into the sarcoplasm.

On the other hand, Bernstein long ago suggested that the changes during muscular contraction are determined by alterations in surface tension. If we consider muscle as made up of a series of chains of oval particles, a chemical change in the surface of these particles, causing an increase of surface tension, will tend to make them assume the globular shape, and will therefore cause a shortening and thickening of the whole fibre.

Or again, the contraction might be due to an alteration in electric charges resulting in attraction or repulsion between surfaces or particles, as suggested by Du Bois Reymond.‡ Such a theory would be a special case of the surface tension hypothesis. In its most modern form this hypothesis is applied to considerations of molecular structure. It is believed that the Q-discs of the sarcomeres are largely composed of rod-like molecules of myosin, which lie with their long axis parallel to the length of the muscle fibre. According to conditions these molecules may form nearly straight chains, or else may be wrinkled in a zig-zag fashion (*v.* p. 49). Such a wrinkling might well be the cause of a shortening of the Q-disc and so cause contraction of the fibre.§

In favour of the hypothesis that the essential factor in the processes of excitation and contraction is an alteration of surface, we may note that the electrical changes

* BENEDICT and CATHCART. Muscular Work. (Carnegie Publ., Washington.) 1913.

† ROAF. *J. Physiol.*, 1911, **43**, xxxviii.

‡ HERMANN'S H'DB'CH. D. PHYSIOL. 1879, I. (1), 245.

§ ASTBURY. *Nature*, 1935, **135**, 95. *Trans. Farad. Soc.*, 1933, **29**, 193.

accompanying the excitatory process denote a depolarisation or diffusion of ions on the surfaces situated in the excited area. The electrical change which is responsible for the current of action at the excited spot takes place almost instantaneously and disappears somewhat more slowly. It would seem that the excitatory process consists essentially in the migration of certain ions from the surface or surfaces in the contractile tissue, and that the passing away of the excitatory state is due to the reappearance of these ions, either by diffusion from the surrounding fluid or by the expenditure of energy derived from chemical changes such as oxidation. A study of the development of tension and of heat production in a muscle on excitation has shown that in both cases the yield of energy on excitation is, within limits, increased by lengthening and diminished by shortening the muscle. Now alteration in length of the muscle will not alter its volume but will alter the extent of its longitudinal surfaces, and it appears therefore that the production of heat as well as of mechanical energy is not a volume but a surface effect.

There are still other theories.

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CHAPTER XI

PLAIN MUSCLE AND OTHER CONTRACTILE TISSUES

OUR knowledge of the physiology of unstriated (also called smooth, plain, or involuntary) muscle is derived from experiments on viscera or organs in which it predominates, *e.g.* the uterus, arteries, stomach, the intestine, ureter, bladder and retractor penis.* This tissue differs from voluntary muscle in containing numerous plexuses of nerve fibres (non-medullated) and ganglion cells, so that in all our researches it is difficult to be certain whether some results are due to the muscle fibres themselves, or to the nerves and nerve cells which are so intimately connected with them ; especially as we have as yet no convenient drug like curare by aid of which we might discriminate between action on muscle and action on nerve.

A further difficulty is that the physiological properties of plain muscle differ with its source, so that it is unsafe to generalise from the results of specific experiments with tissue from only one source. At the one extreme, smooth muscle preserves many of the properties of primitive protoplasm, while at the other it presents properties which indicate a differentiation of function at least as striking as in striated muscle. In general, however, it is distinguished from voluntary muscle not only by its histological structure, in its chemistry, its innervation, and its physical properties, but especially by the phenomena of independent tonus and rhythmic contraction.

The individual fibres of plain muscle are fusiform cells with an oval centrally placed nucleus ; the cytoplasm in the living cell appears to be structureless, and there is a delicate cell wall. The cells are probably connected together by fine intercellular bridges to form a syncytium, by which in all probability the excitatory process can be transmitted from cell to cell, though under conditions which are at present unknown.

The most striking differences in chemical composition between striated and plain muscle are that the latter contains much less soluble protein, only very small quantities of creatine (and phosphagen), smaller amounts of glycogen, less potassium and phosphate and more sodium than the former.

INNERVATION. Skeletal muscle is entirely dependent for its activity on the central nervous system, through the intermediation of the motor nerves. When the nerves to such a muscle are cut it is flabby and motionless and soon atrophies.

In the case of smooth muscle, there is a double nerve supply, one variety being motor, and causing contraction, the other kind inhibitory, and causing cessation of a previous contraction. All these fibres belong to the autonomic system, *i.e.* they arise from ganglion cells outside the central nervous system, and only receive impulses from the central nervous system through the intermediation of these ganglia. Usually one supply is sympathetic, and the other parasympathetic, *e.g.* to the intestinal muscle, sympathetic and vagus : their ultimate ramifications in the muscle form a plexus, and are non-medullated, and not infrequently the ganglion cells from which they arise are also contained in the nerve net near to or among the plain muscle cells which they supply. It is believed that both sources of supply are present in the nerve plexus,

* The *retractor penis*, found in the dog, cat, horse, hedgehog (but not in rabbit or man), is a thin band of longitudinally arranged unstriated muscle, which is inserted at the attachment of the prepuce and is continued backwards in a sheath of connective tissue to the bulb, where it divides into two slips, which pass on either side of the anus. It is innervated from two sources, the *motor* fibres being derived from the lumbar sympathetic and running to the muscle in the internal pudic nerve, while the *inhibitory* fibres run in the pelvic visceral nerves (*nervi erigentes*) and are derived from the second and third sacral nerve roots. (LANGLEY and ANDERSON. *J. Physiol.*, 1895, 19, 88.)

e.g. both vagus and sympathetic fibres are present in the plexus of Auerbach in the intestine.

The physiological significance of the double supply is evident from the fact that, even when deprived of all nerves, plain muscle may exhibit either sustained spontaneous contractions, known as tonic, or else intermittent rhythmic contractions, so that control of both contraction and relaxation is feasible (Fig. 90). In some invertebrates many voluntary striated muscles also possess a double nerve supply, one causing contraction of the relaxed muscle, and one causing relaxation of the contracted muscle (Fig. 91).

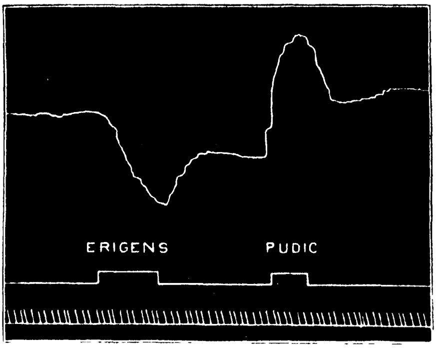

FIG. 90. Tracing from the *Retractor Penis* Muscle of the Dog, showing lengthening (inhibition) on stimulation of the nervus erigens, and a smart contraction on stimulating the pudic (motor) nerve. (Movements of muscle reduced ½.)

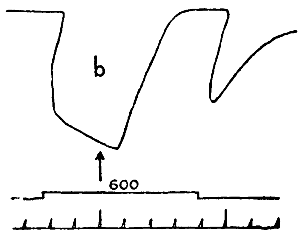

FIG. 91. Tracing of Contraction of Adductor Muscle of Claw of Crayfish, showing inhibition resulting from stimulation of its nerve (at *b*) by means of a constant current. The break of the current causes a second smaller inhibition. (BIEDERMANN.)

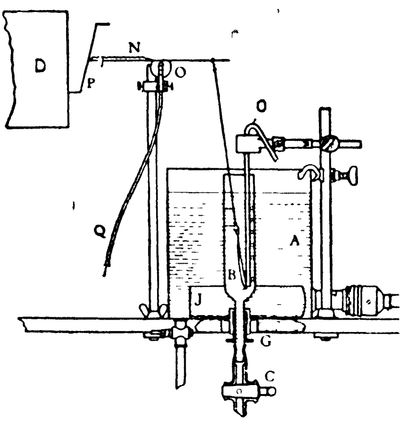

FIG. 92. Thermostat Bath for Investigation of Plain Muscle. A = copper water-bath, kept warm by the lamp J. B = Glass vessel filled with warm saline which can be changed by the tap C. O = the oxygen tube, at the bottom of which is a platinum pin with which the tissue is transfixed. N = a frontal writing lever, to which the tissue is attached by a hair. O, Q = a small brake for fixing the lever while the fluid is changed in B. D = recording drum. (BURN and DALE.)

PHYSIOLOGICAL PROPERTIES. In studying the responses of plain muscle it is not customary, as with amphibian skeletal muscle, to use the moist tissue suspended in air, because under these conditions the tissue behaves irregularly, but, instead, to suspend it in a bath of saline solution of suitable composition, *e.g.* Ringer's fluid, which is kept at the proper temperature and oxygenated with a constant stream of oxygen bubbles. A suitable arrangement is shown in Fig. 92.

Tonus. Plain muscle, isolated in this manner, is very readily extensible, so that when a small weight is applied to it the tissue may extend to several times its former length; the effect of the weight will depend, however, on the degree of *tonus* which the preparation has, and this varies with the source.* Thus the cat's uterus is highly tonic and requires a much larger weight to stretch it than does a guinea-pig's uterus of the same size. Many drugs have the property of affecting the tonus of plain muscle, perhaps by exciting the sympathetic or parasympathetic nerve structures contained within it. Thus adrenaline will cause the uterus to relax (if tonic), while acetyl choline even in high dilutions (*e.g.* 1 part in 10 million of saline) or histamine causes intense tonic contraction (Fig. 93, **3**). Adrenaline in many other respects reproduces the action of the sympathetic nerves, and is therefore called *sympathicomimetic*, while acetyl choline reproduces the action of the parasympathetic nerve supply.

The maintenance of the sustained tonus is apparently not associated with increased expenditure of energy,† for the tissue, while in this condition, uses no more oxygen than when in the relaxed state, even though it may be exerting quite considerable tension; consequently no signs of fatigue are manifested. Thus in the bivalve mollusc Pecten, there is a large striated adductor muscle which can close the shell rapidly but then relaxes, and a smaller smooth muscle whose office it is to hold the two valves together. The weight required to separate the valves, when this latter muscle is contracted, is many times that which will prevent the closure of the shell when open. Thus the smooth adductor muscle can support a weight which it cannot raise. This "catch action" is under the control of the central nervous system by two nerves from the cerebral ganglion. Stimulation of one of these causes contraction, and the muscle remains contracted even after the nerve is divided. Stimulation of the other nerve causes the muscle to relax, so that the shell opens. Apparently the action of these nerves is to allow the length of the muscle to alter, and it then remains at that length until the other nerve is stimulated. Somewhat similar phenomena may be observed in the smooth muscle of higher animals, and it may be that most smooth muscles are capable of two kinds of shortening, one followed by passive relaxation and the other by fixation at a diminished length without the expenditure of extra energy.

Although plain muscle can execute contractions for some time after being completely deprived of oxygen, and although, when in the tonic state, the muscle uses no more oxygen than when it is relaxed, yet the presence of oxygen is essential for the maintenance of tonus (Fig. 93, **5**). It would seem as though oxygen, though not essential for the act of shortening, plays some part in maintaining the integrity of the cell structures which are concerned in fixing the tissue at the shorter length. (GARRY.)‡

Rhythmic Contractions. Most varieties of plain muscle when slightly stretched normally exhibit rhythmic contractions at fairly regular intervals, both in the body and when isolated. The strength and frequency of these rhythmic contractions vary very much according to conditions; in some types of plain muscle, *e.g.* that of the small intestine, they are quite regular, frequent and powerful (Fig. 93, **1**), whereas in other cases they are slow (Fig. 93, **2**), often irregular, and of variable amplitude, being occasionally negligible.

There is strong evidence that they originate in the plain muscle cells themselves, *i.e.* they are myogenic; for example, the cells in the chick amnion, which are devoid of

* WINTON. *J. Physiol.*, 1930, **69**, 393.
† LOVATT EVANS. *J. Physiol.*, 1923, **58**, 22.
‡ GARRY. *J. Physiol.*, 1928, **66**, 235.

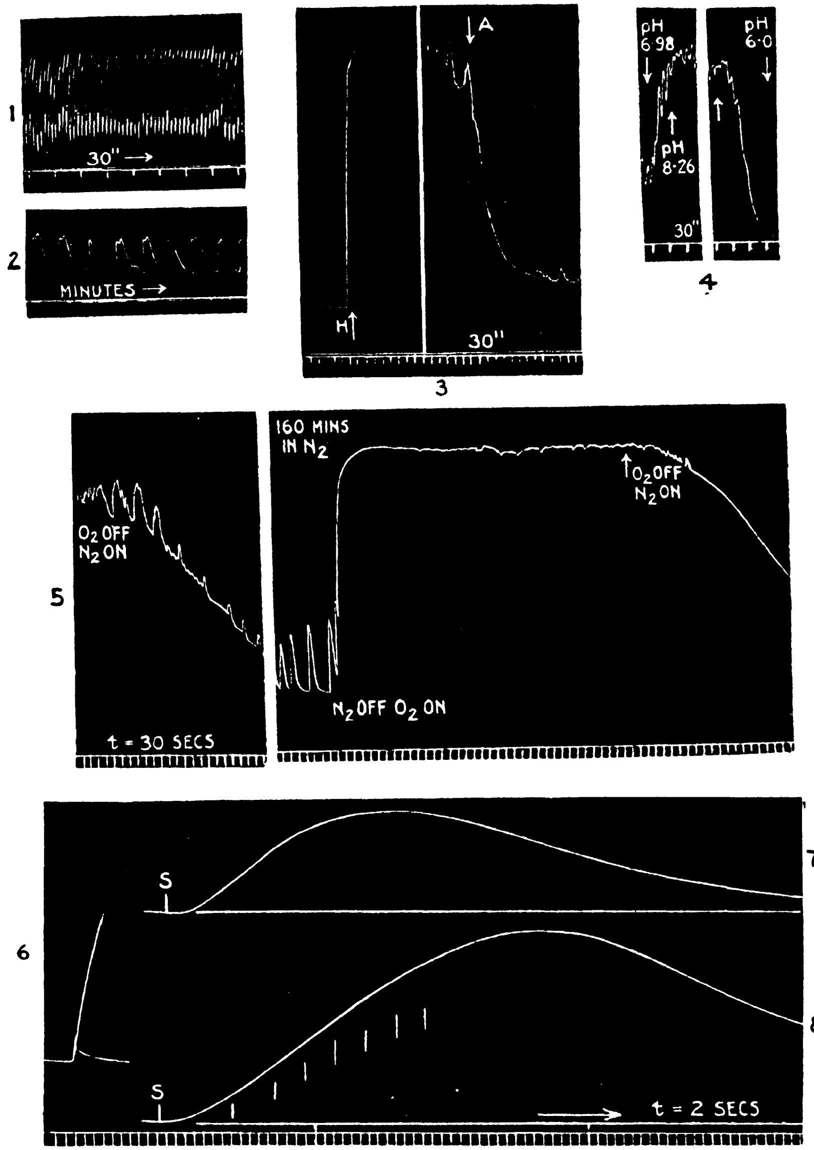

FIG. 93 1. Isolated jejunum of rabbit, showing rhythmic contractions. Time tracing = 30 seconds.

2. Slow rhythmic contractions of guinea-pig uterus. (Time, same scale as 1.)
3. Guinea-pig uterus. At H, histamine 1 in 1 million; at A, adrenaline, 1 in 4 million. Time = 30 seconds.
4. Small intestine of cat, showing contraction on raising, and relaxation on lowering the *p*H of the fluid in the bath. Time = 30 seconds; *p*H measurements taken at arrow. (EVANS and UNDERHILL.)
5. Uterus of cat, showing effect of oxygen lack caused by replacing oxygen bubbles by nitrogen. The second portion is taken after about two and a half hours in nitrogen.
6. On left, contraction of frog's gastrocnemius to compare with contraction of frog's stomach (7) on same slow drum. Point of stimulation = S. 8. Summation of contraction in frog's stomach. The first stimulus is followed by seven others at the points shown.

nerves, show these rhythmic contractions; further, strips of the inner muscular coat of the small intestine, also free from nerve structures, were found by Gunn and Underhill * to exhibit rhythmic contractions.

Stimulation. Smooth muscle responds to very various stimuli—electrical, mechanical, thermal, light, chemical, &c. As we should expect, however, from the sluggish response of this kind of contractile tissue (high chronaxie), the optimum rate of change of current which excites is very much slower than in the case of striated muscle. Thus in many instances a single induction shock is powerless to excite contraction. A better stimulus is the make or break of a constant current. When the latter form of stimulation is used, response occurs at the make sooner than at the break and, as in other tissues,, the make excitation starts from the cathode and the break excitation from the anode. Nerve-muscle preparations of mammalian plain muscle (stomach) with either or both of the nerves may be made.† Stimulation of the sympathetic supply with slow shocks usually caused contraction; with more rapid stimuli relaxation was the rule. Stimulation of the vagus supply caused contraction. When sympathetic stimulation was applied during contraction due to vagal stimulation, the muscle relaxed, though the same stimulus to the sympathetic alone caused contraction (Fig. 94).

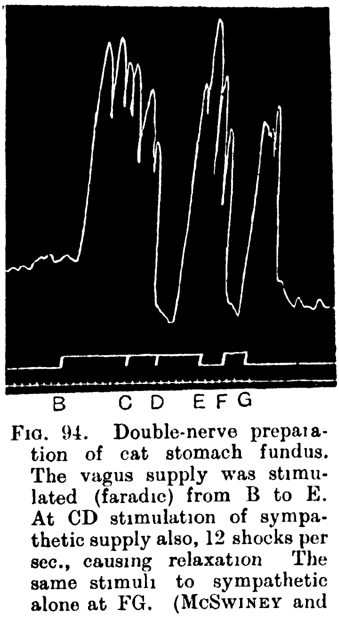

FIG. 94. Double-nerve preparation of cat stomach fundus. The vagus supply was stimulated (faradic) from B to E. At CD stimulation of sympathetic supply also, 12 shocks per sec., causing relaxation The same stimuli to sympathetic alone at FG. (MCSWINEY and ROBSON.)

The contraction curve of plain muscle, though very variable, is much slower than that of voluntary muscle, and the phenomena of latent period, shortening, and relaxation can easily be followed with the eye. The latent period may be from 0·2 to one or two seconds, and the period of contraction from half a second to a few minutes, with proportional durations of relaxation (Fig. 93, 7); the stronger the stimulus the shorter the latent period and the higher the contraction. According to Roos‡ the real latent period of plain muscle is much shorter than is generally supposed, being only 40 σ for frog stomach. The reason for the apparently long latent period, according to him, is that the onset of contraction is so slow that it is not detectable by the levers commonly employed. He regards the electrical variation and the mechanical change as occurring simultaneously.

As in voluntary muscle, if one stimulus follows another at a sufficiently short interval, we may have an increased liberation of energy due to repetition of the stimulus during the rise of the excitatory condition (*summation of contractions*) (Fig. 93, **8**). As might be expected the refractory period is also longer in involuntary muscle.

In all involuntary muscle we may observe summation of the effects of stimuli, when each individual stimulus is insufficient to produce any excitation. Thus we may find a strength of induction shock which, applied singly, is just insufficient to evoke any response. If, however, the shocks are repeated at intervals of a second, it will be found that the first three or four

* GUNN and UNDERHILL. *Quart. J. Exp. Physiol.*, 1914, **8**, 275.
† MCSWINEY and ROBSON. *J. Physiol.*, 1929, 6[illegible], 124; 1931, **71**, 194; **73**, 141.
‡ ROOS. *Arch. néerl. de physiol.*, 1932, **17**, 57.

stimuli are ineffective, and then the muscle enters into a contraction which increases with each succeeding stimulus until it has attained its maximum. There is thus *latent addition* of excitation. Each stimulus, in fact, alters the state of the contractile tissue and makes it more ready to respond to the next stimulus.

Latent addition is a general phenomenon of excitation, but appears to be of special importance in plain muscle. A plain muscle fibre may receive several nerve fibres and may only respond after the effects of several nerve impulses have been summated.*

It has been shown by Brocklehurst † and by Winton ‡ that, as with other forms of muscle, the tension developed on contraction by plain muscle increases with increase of initial length, a very important property in view of the common occurrence of plain muscle in the walls of hollow viscera, where it is subjected to various degrees of extension

CHEMICAL STIMULATION. The tissue is very sensitive to alterations in the hydrogen ion concentration; within limits, lowering of H-ion causes contraction or tonus, with increased rate of rhythmic contractions,§ while increase causes the reverse (Fig. 93, **4**). Many drugs, such as eserine, salts of lead and barium, histamine, acetyl choline, pilocarpine, may cause contraction. As one would expect, however, from the greater independence of the smooth muscle, the action of these drugs varies from organ to organ, muscle fibres apparently histologically identical reacting diversely according to their origin. Some of these drugs, *e.g.* eserine, barium salts and perhaps pituitrin, appear to act directly on the muscle cells, while others, *e.g.* adrenaline, exert their action on the accompanying nerves or their endings; accordingly, adrenaline may cause either relaxation (*e.g.* of intestine) or contraction (*e.g.* of retractor penis).

The question arises whether nervous stimulation may not produce its effects by the liberation of active chemical substances at the nerve terminations, or in the nerve network. Extracts of plain muscle certainly contain substances which can cause other plain muscle to contract,|| and the active constituent in these extracts is probably acetyl choline.¶ It has been shown also that when plain muscle is made to contract by stimulation of nerve, substances are set free which can cause other plain muscle to contract. The action of these substances, like that of acetyl-choline, is enhanced by eserine and counteracted by atropine.

Similar relations have been found by Finkleman** and by Cannon and his associates †† for sympathetic stimulation. When the sympathetic supply to almost any plain muscle is stimulated an adrenaline-like substance which Cannon has called "sympathin" is liberated and may enter the blood stream, in which case it causes acceleration of the denervated heart and other manifestations of sympathetic activity.

MECHANICAL STIMULATION. Smooth muscle is very sensitive to mechanical stimuli, and may react with a local or a general (propagated) contraction. One form of mechanical stimulation is that produced by tension. The effect of increasing the tension on smooth muscle may be twofold: in the first place extension and in the second excitation with increased contraction. These two effects are illustrated in the bladder. If this

* ECCLES and MAGLADERY. *J. Physiol*, 1937, **90**, 31.

† BROCKLEHURST. *J. Physiol.*, 1926, **61**, 275.

‡ WINTON. *Ibid.*, p. 368.

§ LOVATT EVANS and UNDERHILL. *J. Physiol*, 1923, **58**, 1; MCSWINEY and NEWTON *J. Physiol.*, 1927, **63**, 51.

|| LE HEUX. *Pfluger's Arch.*, 1918, **173**, 8; 1920, **179**, 177, 1921, **190**, 280.

¶ DALE and DUDLEY. *J. Physiol.*, 1929, **68**, 97.

** FINKLEMAN. *J. Physiol.*, 1930, **70**, 145.

†† CANNON and BACQ. *Amer. J. Physiol.*, 1931, **96**, 392; ROSENBLUETH and CANNON. *Amer. J. Physiol.*, 1932, **99**, 398.

has all its connections with the central nervous system severed, it is when empty in a state of tonic contraction. If fluid be injected into it rapidly, there is a great rise of pressure in its cavity due to the forcible distension. If, however, the fluid be injected slowly, the bladder muscle relaxes to make room for it, so that a considerable amount of fluid may be accommodated in the bladder without any great rise of pressure. If the injection of fluid be continued, the walls begin to be stretched passively, and this increased tension acts as a stimulus causing rhythmic contractions of the whole bladder.

A peculiar property of plain muscle is that a stimulus (*e.g.* electrical or mechanical) which, when applied to the relaxed muscle, causes a contraction, will, when applied to the tonically contracted muscle, often provoke a rapid relaxation, and *vice versa*. The same even holds true in some instances for the effects of stimuli applied to the nerves supplying plain muscle. This property is perhaps responsible for the occurrence of rhythmic contractions when the muscle is given the constant stimulus of a slight stretch ; this alternately excites the extended muscle to contract and the contracted muscle to relax.

ELECTRICAL POTENTIALS. Accompanying the stimulation and response of plain muscle are a series of potential changes. Two are negative potential waves associated with the contraction ; another negative wave is more easily seen on subliminal stimulation and is related to changes responsible for latent addition. After prolonged or powerful stimulation *via* the nerve, or chemically, rhythmic responses, shown by potential undulations, often occur ; in these there is approximate synchronisation of contraction and relaxation in many fibres.

PROPAGATION OF THE EXCITATORY STATE, OR WAVE OF CONTRACTION. On stimulating any part of a voluntary muscle fibre, a wave of contraction is started which travels to each end of the fibre, but no further. There is no propagation from muscle fibre to muscle fibre, the synchronous contraction of the whole muscle being brought about when there is simultaneous excitation of all its fibres. This isolation of the excitatory state is not found in smooth muscle. Frequently a stimulus applied to any part of a sheet of smooth fibres may travel all over the sheet just as if it were a single fibre. It seems probable indeed that there is protoplasmic continuity by means of fine bridge-like processes between adjacent muscle cells.

It is claimed that each fibre of smooth muscle gives an all-or-none response to a stimulus.* Probably the contraction curve as commonly recorded represents the simultaneous or the successive action of the component cells, and a feeble contraction is probably due to the all-or-none response of only a few of them. It is certain for instance that feeble stimuli may produce only local contraction, especially in fatigued tissue. Thus, in the retractor penis of the dog, we get graded contractions from graded stimuli, and it is noticeable that with increasing strength of stimulus a greater extent of muscle enters into contraction. Action potentials are usually proportional to the extent of the contraction, *i.e.*, to the number of fibres simultaneously in action ; but tonic contractions may occur without propagated action potentials † ; in such cases the fibres are contracting asynchronously.

It must be remembered that in all unstriated muscle the fibres are surrounded by a network of non-medullated nerve fibres. Some physiologists are inclined to ascribe to these fibres an important part in the propagation of the contraction wave.

INFLUENCE OF TEMPERATURE. Plain muscle is extremely susceptible to changes of temperature ; as a rule, warming causes relaxation, while application of cold causes a tonic contraction. The condition of the muscle at any given time depends not only on its actual temperature, but also on the rapidity with which that temperature has been reached. Thus a rapid cooling of the retractor penis muscle of a dog from 35° to 25° may cause a contraction as extensive as would be produced by a slow cooling to

* FLETCHER. *J. Physiol.*, 1938, **91**, 172.
† ECCLES and MAGLADERY. *J. Physiol*, 1937, **90**, 31, 68.

5° C. On warming plain muscle from 30° to 50° C. it lengthens gradually up to about 40°, and it may then undergo a marked heat contraction (varying in degree in different muscles) at about 50° C., which may pass off at a somewhat higher temperature. It is first paralysed and then killed somewhere between 40° and 50° C.* It seems very doubtful whether any true rigor mortis occurs in smooth muscle. The hard contracted appearance of the smooth muscle in a recently dead animal is chiefly conditioned by the fall of temperature. On excising the muscle and warming it up to body temperature it may again relax and show signs of irritability two or three days after the death of the animal.

The Chemistry of Contraction in plain muscle, so far as it has been studied, is very similar to that in striated muscle.† Glycogen disappears and lactic acid arises when the muscle contracts, or when it is asphyxiated or treated with arsenates. The action of caffeine is, however, different, for this retards lactic acid formation in plain muscle. During recovery from contraction lactic acid is removed and glycogen restored. The amounts of lactic acid produced and of glycogen removed in contraction are relatively small, as would be expected in view of the small amount of energy it yields.

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CARDIAC MUSCLE. The properties of cardiac, or heart, muscle will be considered in Chapter XXXI.

AMŒBOID MOVEMENT

Amœboid movement is seen in the unicellular organisms such as the amœba and in the polymorphonuclear blood corpuscles. It can occur only within certain limits of temperature (about 0° to 40° C.); within these limits it is the more active the higher the temperature. At about 45° the cell goes into a condition resembling heat rigor.

The fluid in which the corpuscles are suspended is of great importance. Distilled water, almost all salts, acids and alkalies, if strong enough, stop the action and kill the cell.

The movements are also arrested by CO_2 or by absence of oxygen.

Artificial excitation, whether electrical, chemical or thermal, causes withdrawal of the pseudopodia and contraction of the corpuscle, which therefore assumes the spherical form. Electrical stimulation also causes (*e.g.* in *Amœba*) a sudden gelation of the cytoplasm, which sets quite suddenly; ‡ after the stimulus is over, the fluid state returns.

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CILIARY MOVEMENT

Cilia are met with in man in nearly the whole of the respiratory passages and the cavities opening into them, in the generative organs, in the Fallopian tubes of the female and the epididymis of the male, and on the ependyma of the central canal of the spinal cord and its continuation into the cerebral ventricles.

The cilia (Fig. 95) are delicate tapering filaments which project from the hyaline border of the epithelial cells. There may be twenty or thirty to each cell. The hyaline border is really made up of the enlarged basal portions of the cilia and seems to consist of a row of multiple centrioles.

* LOVATT EVANS. *J. Physiol.*, 1921, **54**, Proc. cxxxix.
† LOVATT EVANS. *Biochem. J.*, 1925, **19**, 1115; 1926, **20**, 893.
‡ BAYLISS. *Proc. Roy. Soc.*, 1919, **91b**, 196.

The trachea of a mammal slit open along the back and pinned out under warm oxygenated Ringer's solution forms a suitable object for demonstrating ciliary action If a drop of graphite suspension be placed on the mucous membrane, it will be seen to become entangled in mucus and pushed towards the upper end of the trachea at the rate of 1 to 2 cm. per minute. By this means any dust particles trapped on the ciliated surfaces of the respiratory tract are swept upwards to the mouth.

In action the cilia bend suddenly down into a hook or sickle form, and then return slowly to the erect position. This movement is repeated many (twelve to twenty) times a second, and thus serves to move forward mucus, dust or an ovum, as the case may be.

During the rapid, or effective, phase the cilium appears to be rigid, while during the slower return phase it is limp. No structure is demonstrable within the cilium to account for its power of movement. The minuteness of the object has up to now prevented us from deciding whether the cilium is itself actively contractile, or whether it is passively moved by the action of the basal part situated in the hyaline border of the cell. An attractive suggestion, due to Gray, is that the movement may be due to a rhythmic alteration in the distribution of water in the cilium ; for example, a transference of water to one side would cause that side to swell, and so, like paper wetted on one surface, to bend over. The movement is apparently dependent upon connection of the cilia with their basal granules, since when the ciliated hem is stripped away, movement ceases at once.

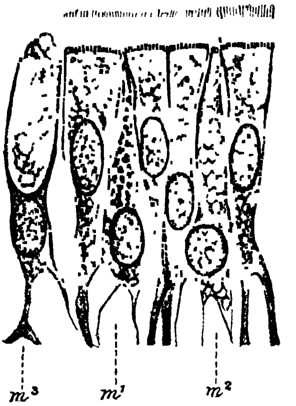

FIG. 95. Ciliated Columnar Epithelium from the Trachea of a Rabbit; m^1, m^2, m^3, mucus-secreting cells. (SCHAFER.)

There seems to be a functional connection between all the cells of a ciliated epithelial surface. The action is passed from cell to cell and from cilium to cilium, in succession. When looked at from above, a series of waves (*metachronal waves*) are seen to pass in regular sequence, over the ciliated surface, just as, when the wind blows, waves of bending pass over a field of corn.

Ciliary movement is not dependent upon nervous action, though in some instances it can be accelerated or inhibited through the action of nerves. Thus Seo claims that the rate of ciliary movement in the palate of the frog is accelerated by stimulation of appropriate nerves which are distributed to the palate and form a nerve-net. By the use of a " nerve-cilia " preparation consisting of the palate and palatine nerve, he finds that the velocity with which red blood cells can be swept along the ciliated surface is accelerated 7–20 times by stimulation of the nerve. This acceleration is due to more rapid metachronal waves, greater speed of effective phase and greater amplitude of movement of cilia. Normally he claims that the regulation of ciliary action is a reflex confined to the same side of the palate as the stimuli.

The conditions of ciliary action are the same as those for amœboid movement of naked cells. Acids or excess of calcium ions slow, and alkalies or potassium ions hasten, the rate of action. Rise of temperature, within limits, hastens, and cold slows, the rate, but over most of these ranges the amplitude of the movement is constant. Oxygen is utilised at a rate proportional to the rate of ciliary action, and though ciliary action will persist for a time in its absence, the movement ultimately fails if it is withheld (Gray).

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CHAPTER XII

NERVE FIBRES

THE STRUCTURE OF NERVE FIBRES

On stimulating the nerve of a nerve muscle preparation at any part, the stimulus is followed after a very short interval by a contraction of the muscle. This observation illustrates the two functions of nerve fibres, excitability and conductivity—that is to say, a suitable stimulus can set up in any part of the nerve a disturbance which is propagated down the nerve without any visible effects occurring in it; and it is not until this *nervous impulse* has reached the muscle that a visible effect is observed in the shape of a contraction. In the animal body a direct excitation of the nerve fibre in its course never takes place under normal circumstances. The only function the nerve fibre has to perform is that of conducting impulses from the sense organs at the periphery to the central nervous system, or efferent impulses from this to the muscles and other effector organs. Hence it is absolutely essential that there should be continuity along the whole length of the fibre. Damage to any part, such as by crushing or heat, causes a block to the passage of an impulse.

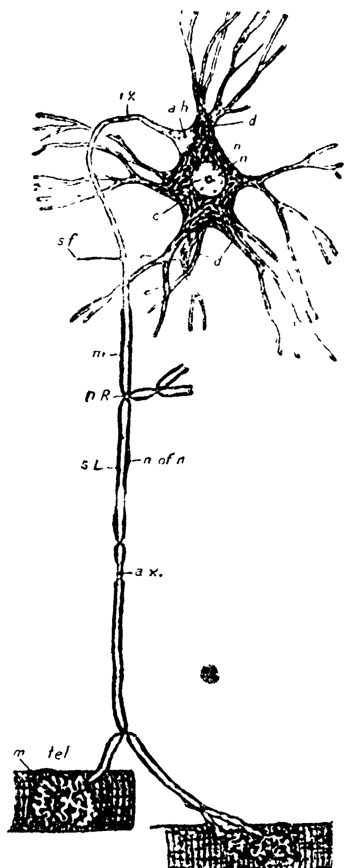

Fig. 96. Diagram of a Motor Nerve Cell with its Nerve Fibre and two motor-end plates. (After Barker.) *a.h*, axon hillock ; *d*, dendrites , *a.x*, axis cylinder ; *m*, medullary sheath ; *n.R*, node of Ranvier.

A nerve fibre is essentially a long process of a nerve cell (Fig. 96). The cell is in the higher animals usually withdrawn from the surface into a special collection of cells such as the posterior root ganglion, or may be one of the mass of cells and fibres making up the central nervous system. All nerves are alike in possessing as their conducting part the continuous strand of protoplasm produced from the nerve cell and known as the *axon* or *axis cylinder*. In the fresh condition the axon appears to be a homogeneous fluid, though after fixation special staining methods bring out the presence of *fibrillæ* or *neuro-fibrils*, embedded in a clear material, the *axioplasm* (Fig. 97). There is a difference of opinion as to the existence of neurofibrils in the living nerve fibre. In the giant nerve fibres of Cephalopods, which may reach 1 mm, in diameter, the contents are fluid and run out when the fibre is cut

across.* But micro-dissection of living nerve fibres in other invertebrates and also in vertebrates shows the contents of the axis cylinder to be gelatinous and to contain neuro-fibrils as tough threads.†

In the course of growth the nerves develop certain histological differences, which appear to bear some relation to the nature of the processes they conduct or to the character of their parent cell. Thus all the fibres which are given off from and which enter the central nervous system, *i.e.* the brain and spinal cord, belong to the class known as *medullated.* In this type the axon is surrounded with a layer of material known as *myelin,* forming the *medullary sheath.* This sheath consists of a fatty material composed largely of lecithin, and staining black with osmic acid, supported in the interstices of a network formed of a horny substance known as *neurokeratin.* The medullary sheath is surrounded by a structureless membrane, the primitive sheath or *neurolemma.* At regular intervals a break occurs in the medullary sheath, the neurolemma coming in close contact with the axis cylinder which is constricted at that place. This break is the *node of Ranvier,* the intervening portions of medullated nerve being the *internodes.* In each internode, lying closely under the neurolemma, is an oval nucleus. The medullated nerve fibres vary considerably in diameter, the largest fibres being distributed to the muscles and skin, the smallest carrying impulses from the central nervous system to the viscera (Fig. 98).

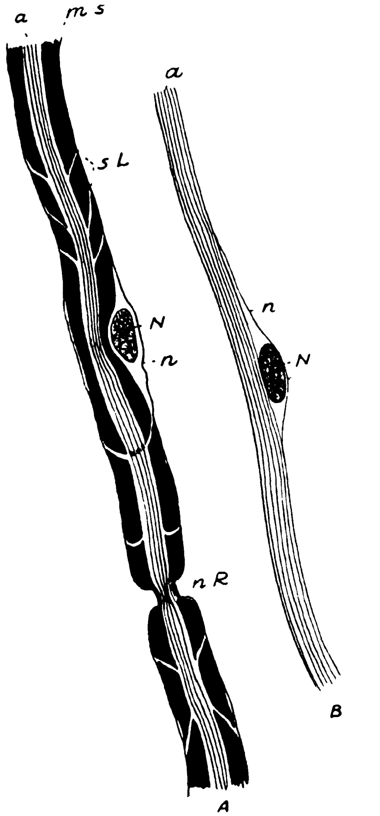

FIG. 97. A, portion of a medullated nerve fibre a, the axis-cylinder, containing neurofibrils, *m.s.,* the medullary sheath; *n,* the neurolemma; N, a nucleus, *n* R, a node of Ranvier; *s.*L, segments of Lantermann (artefacts); B, portion of a non medullated fibre. Lettering as for A.

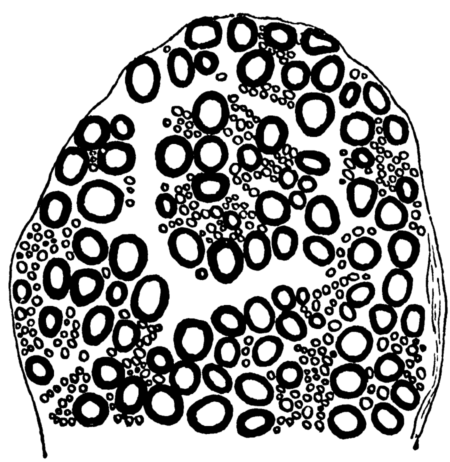

FIG. 98. Transverse section of Second Thoracic Ventral Nerve Root of Dog, showing varying size of the fibres. (Gaskell.)

The latter all come to an end in some collection of ganglion cells of the sympathetic chain or peripheral ganglia, the impulses being carried on to their destination by a fresh relay of non-medullated nerve fibres.

The medullated nerves are bound together by connective tissue (*endoneurium*) into small bundles, which are again united by tougher connective tissue into larger nerve trunks. The fibres as a rule branch only when in close proximity to their destination, and then the branching always occurs at a node of Ranvier. The number of branches may be large; thus each motor fibre which arises from a motor

* YOUNG. *J. Physiol.,* 1935, **83,** 27P.
† DE RENYI. *J. Comp. Neurol.,* 1929, **48,** 441.

cell may supply as many as 150 muscle fibres, and the name *motor-unit* is given to this collection of muscle fibres, together with its motor nerve fibre. Each motor unit is activated by one nerve cell, from which the motor fibre is a process. In this manner a whole muscle may be under the control of relatively few motor nerve cells and motor nerve fibres—*e.g.* the soleus of the cat has 30,000 muscle fibres grouped into 230 motor-units, *i.e.* its motor nerve contains only 230 fibres.

The *non-medullated fibres* (Fig. 97B) differ from the medullated simply in the absence of a medullary sheath. They possess a neurolemma, under which we find nuclei lying closely on the side of the fibre and bulging out the sheath. In their ultimate ramifications they tend to form close networks or plexuses and appear to lose the last traces of a sheath.

As to the functions of the myelin sheath in the medullated nerve fibre very little is known. It does not make its appearance until the axis cylinder is formed, and is apparently derived from a series of cells which grow out from the spongioblasts of the central nervous system and form a chain surrounding the out-growing axons. In the regeneration of a nerve fibre after section, the myelin sheath appears later than the axon in the peripheral part of the nerve. It has been supposed by some to act as a sort of insulator ensuring isolated conduction within any given nerve fibre.

THE DEGENERATION OF NERVE FIBRES. When an axon is severed, either by actual division, or functionally by localised damage by chemical or physical means, and so cut off from the cell upon which its life depends, the part so cut off undergoes rapid degenerative changes. For a few millimetres on either side of the cut there are acute changes known as *traumatic degeneration*. But the peripheral portion also shows, simultaneously along its whole length,* the changes of secondary or *Wallerian degeneration*, which commence in the first hour and progress to complete degeneration in a few days; loss of excitability to stimuli is complete after three days. The central portion of the fibre does not undergo secondary degeneration, though it may atrophy later on (retrograde degeneration) as may the cell body, if no fibre regeneration takes place.

The structural changes during degeneration are initiated by the appearance of swellings and fusion in the neurofibrils and shortly afterwards the axis cylinder breaks up irregularly into short lengths. The myelin sheath, in medullated nerves, breaks up into irregular droplets and undergoes chemical changes, among which is the liberation of highly unsaturated fatty acids. In consequence of this the degenerating medullated nerve fibres are stained black when treated with Marchi's method, a fact of great importance for mapping out the tracts of the central nervous system. Meanwhile the cells of the neurolemma rapidly proliferate. Lastly, the proliferated neurolemma cells, together with histiocytes of the surrounding connective tissue, digest and absorb the remnants of the axis cylinder and myelin sheath, so that all that ultimately remains of the fibre is a thin thread consisting of modified neurolemma cells. The specialised terminal nervous structures such as motor end-plates, sensory endings, etc., also undergo degeneration.

REGENERATION OF NERVE FIBRES. Some time after section and degeneration, a process of regeneration may occur in certain nerves. The process occurs by the formation of outgrowths of neurofibrils from the undegenerated stumps which remain connected to their cells. These outgrowths have bulbous ends, and sprout down, probably by some sort of chemiotactic process, into the neurolemmal remains of the degenerated fibre, growing at a rate of about 0·5 to 2 mm. per day. At the same time, if there is a gap, the cells of the old neurolemma proliferate and grow up until they establish continuity with the neurolemma of the fibres in the normal

* SUGAR. *J. Neurophysiol.*, 1938, **1**, 7.

stump. The presence of the old track, preserved by the neurolemma cells, appears to be indispensable for the proper guidance of the growing axons. Hence, if the gap is great, the bulbous ends of the axons grope about in the tissues, and end by forming a tangled mass, or neuroma. Because of the necessity for the neurolemma also, regeneration never takes place in those fibres of the nervous system that have no neurolemma, *e.g.*, in the white matter of the central nervous system, the optic nerve, or the sensory root of the Vth cranial nerve.

After the establishment of contact of the growing ends of the fibres with the old neurolemma tracks, growth steadily proceeds along these until, the terminations having been reached and regeneration of specific end-structures effected, re-establishment of function becomes possible. The medullary sheath is reformed shortly after the regeneration of the axis cylinder. The extent to which functional recovery takes place, and also its speed, varies greatly with the conditions attending the section, and with its site. If the division is a purely functional one, as by local crushing, recovery is relatively rapid, especially if the length to be regenerated is a short one. Moreover, the degree of recovery of function is considerable, because each new fibre has a fair chance of growing into its former track.* If the nerve is actually severed and the cut ends sutured together there will be less rapid and less complete recovery of function ; it has been found that a fuller recovery ensues in this case if the two ends are not brought together for a week or two, so that preliminary degeneration is complete before reunion is encouraged. If a considerable gap is left between the two divided ends recovery is very slow and often but slight.

These incomplete recoveries are ascribed to the fact that the nerve fibres, when they grow, enter the tracks of fibres of different function.† Thus in a mixed nerve, a sensory axon may grow down into the track of a motor fibre, and no functional result would follow. Or a sensory fibre, say from skin, may grow into another skin sensory track of different function, and in that case abnormal sensations would arise, for the removal of which re-education over a considerable time would be needed.

The reunion of motor nerves of different muscles has found application in surgery. Thus the central end of the divided spinal accessory has been united to the distal end of a paralysed facial nerve, and after regeneration and re-education has given good results.

Langley and Anderson ‡ also united many different nerves, *e.g.*, the central end of the vagus with the peripheral end of the cervical sympathetic and showed that the vagus fibres regenerated up to the synapses of the superior cervical ganglion. As a result of their researches, moreover, it is clear that what are now called cholinergic nerves cannot be united to adrenergic nerves.

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THE EXCITATION OF NERVE FIBRES

METHODS. Many different forms of stimuli, such as thermal, mechanical or chemical stimuli may be used to arouse the activity of nerve. Sudden warming of the nerve always gives rise to excitation. At about

* SHARPEY SCHAFER. *Brain*, 1927, **50**, 538.
† STOPFORD. "Sensation and the Sensory Pathway." London. Longmans Green. 1930.
‡ LANGLEY and ANDERSON. *J. Physiol.*, 1904, **31**, 365.

45° C. the nerve dies. A nerve may be rapidly cooled without any excitation taking place.

A nerve may be excited mechanically by crushing or cutting, or by carefully graduated taps. The chemical stimuli most used are strong salt solutions, glycerol or weak acids.

None of these forms of stimuli can be adequately controlled either as to strength or duration, and all cause some damage. We therefore use electrical stimuli. For this purpose we may use either the make and break of a constant current, the induced current, or the discharge of a condenser. Alternating currents also provide an excellent method for stimulating nerves ; any desired current strength or frequency can be obtained. The frequency (expressed in Hertz, *i.e.*, cycles per second) best suited for stimulation may vary considerably.

The relation of current strength and duration to excitation in nerve fibres is determined by methods similar to those used for muscle, and strength-duration curves having similar significance can be plotted, as already discussed. A nerve cannot be excited by currents passed transversely across it, the threshold for stimulation being inversely proportional to the cosine of the angle between current and nerve (cos 90° = 0).

CRITERIA OF EXCITATION. As evidence that excitation (and conduction) has taken place we have the effects produced (*e.g.*, in the muscle in the case of a motor nerve) at the tissue supplied, and also the passage of a wave of electrical disturbance along the nerve.

The Electric Potential. If we connect a high resistance galvanometer to two points of an uninjured nerve, no current is observed, because the whole surface is polarized, so as to be positively charged at the surface (*v.* Chapter VIII). On making an injury, *e.g.*, with a hot wire, to the nerve at one leading-off point, a continuous current passes from the uninjured lead-off point through the galvanometer to the injured spot. This ‘ injury current ’ is due to the fact that at the injured place the negatively charged inner surface of the polarized membrane is accessible. As time passes, this current rapidly diminishes in strength and finally disappears.

When a nerve is stimulated so as to cause excitation, a transitory state of negativity results at the excited point, as occurs in a muscle, and this state of negativity travels as a wave at a definite velocity in both directions along the fibres, to be followed by recovery processes which restore the tissue to the resting state. This travelling disturbance is a convenient objective indication of the passage of a nervous impulse, and, in fact, *is* the nervous impulse.

It should be made clear to beginners that when we say that an electrical disturbance travels along the nerve, this does not mean that electricity travels along the nerve, any more than if we carry an accumulator across a room it means that electricity travels across it. An electric current can be conducted through a nerve cooled to 0°, but a nervous impulse cannot pass in such a cooled nerve.

Usually each stimulus causes a single nerve impulse, though very powerful ones may cause two or three in succession (repetitive response). Lack of calcium increases the tendency to repetitive response.*

In order to demonstrate the wave-like progression of the electrical change from an excited spot along the nerve, it is now customary to amplify the waves by thermionic valves and to record by means of a cathode ray or other oscillograph (Fig. 86). If we lead off from an injured and an uninjured spot, the passage of each impulse past the uninjured spot will give rise to a

* KATZ. *J. Physiol.*, 1937, **88**, 239.

monophasic variation; if both spots are uninjured the variation will be diphasic (Fig. 89). The amplitude is about 25 to 50 millivolts in the fibre. The change progresses along the nerve at the same rate as the nervous impulse, *i.e.*, about 100 metres per second in the mammalian A-fibres, and lasts only an extremely short interval of time at each spot; thus the 'rising phase' in mammalian A-fibres occupies 0·2 msec. and the total duration 0·4 msec.

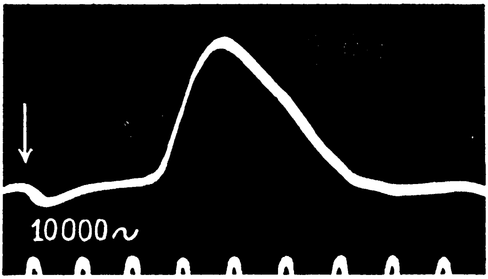

FIG. 99. Spike from single mammalian nerve fibre. Stimulus at arrow. The latent period is made up of true latent period at cathode plus conducting time to the lead. (GASSER, *J. Appl. Physics*, 1938, **9**, 88.)

(Fig. 99), the figures for frog being 0·3 msec. and 0·9 msec. respectively.* Thus the *length* of the excitatory wave in mammalian A-fibres is about 40 mm.

This large monophasic wave is what is generally recorded as a means of recognising the passage of nervous impulses along nerve fibres under any given conditions of experiment, and is now referred to as a '*spike-potential*' to distinguish it from smaller and slower potential changes which modern refinements of technique have shown to follow it.

A

B

C

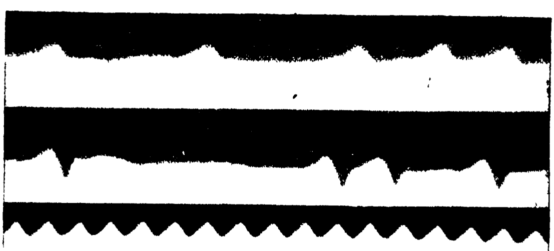

FIG. 100. Records by Oscillograph of Action Potentials produced in frog's nerve by repeated stimulation.
A = nerve injured (monophasic).
B = nerve intact (diphasic).
C = time trace 200 d.v. per second. (MATTHEWS.)

These are respectively the *negative and positive after-potentials*.† (Fig. 101) In mammalian fibres the positive after-potential cuts into the preceding negative after-potential. In the case of the A-fibres the spike lasts about 0·4 msec.; the negative after-potential continues the fall of negative potential, though more slowly, and may last 15 msec.; the positive after-potential is only about 0·2 per cent. of the magnitude of the spike, but lasts till 70 msec., and may then be succeeded by another negative after-potential. Tetanic stimuli (Fig. 102) or application of veratrine increase both after-potentials.

* GASSER and ERLANGER. *Amer. J. Physiol.*, 1927, **80**, 522.
† GASSER and GRUNDFEST. *Amer. J. Physiol.*, 1936, **117**, 113.

For C fibres the spike lasts 2 msec., the negative after-potential up to 80 msec., and the positive after-potential for 1 to 2 seconds, and is large (1·5 per cent of spike).

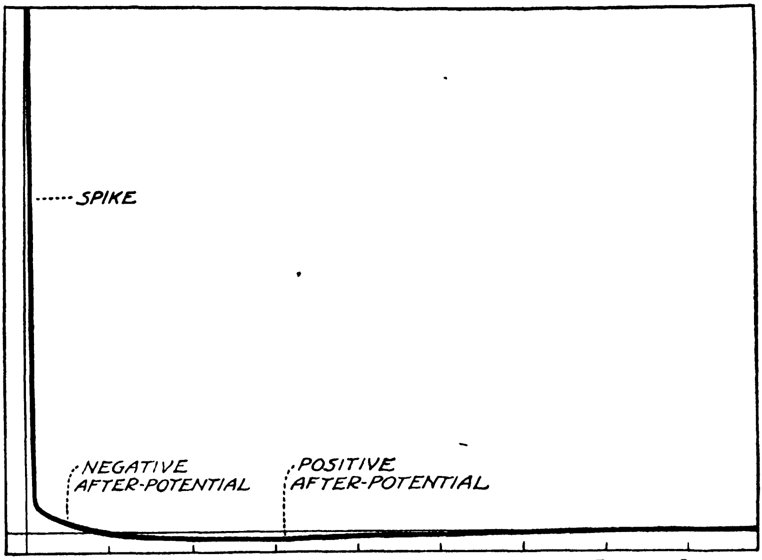

FIG. 101. Diagram of Electrical Potentials in Nerve following Stimulation at 0. The positive after-potential has been exaggerated. (After GASSER. Harvey Lect., 1937.)

The absolute refractory period in a nerve fibre occurs during the spike, the relative refractory phase is short and is followed by a supernormal phase lasting till the end of the negative after-potential, while the succeeding subnormal phase coincides with the positive after-potential.* It seems clear therefore that the after-potentials are an indication of the level of excitability, whereas the spike is the actual message carrier.

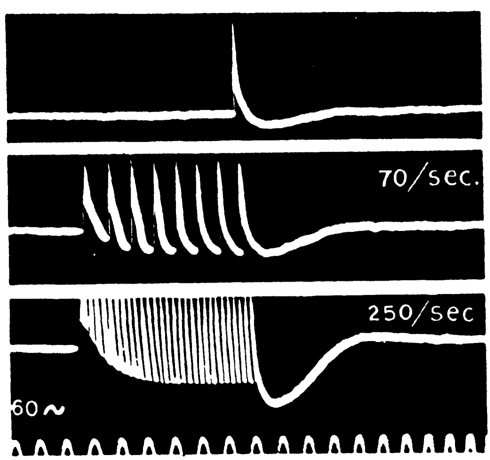

FIG. 102. Action and after-potentials in Phrenic Nerve, from single stimulus, from 8 stimuli at 70/sec., and from num(rous stimnli at 250/sec. Note increase in positive after-potentials (dip below zero line) as frequency in- creases. (GASSER. J Appl. Physics, 1938, 9, 88)

When by repeated stimulation the nerve is thrown into protracted activity, a study of the spike potentials indicates that the excitatory changes in the nerve fibre are *interrupted*, not continuous. We may compare the passage of such a stream of nerve impulses to the discharge of a stream of machine-gun bullets, rather than to the flow of a stream of water; 'continuous' activity of the fibre being represented by a quick succession of these short waves (Fig. 102). It has been shown that the normal

* GRAHAM, HELEN T. *Amer. J. Physiol.*, 1935, **111**, 452.

impulses travelling up sensory nerves are of similar nature to those which normally travel down the motor nerves.

The maximum frequency at which the impulses can follow one another is determined by the length of the refractory period, since a second response cannot be obtained until the previous one has subsided. Since the absolute refractory period for mammalian A-fibres is about 0·4 msec., it follows that the maximum frequency is about 2500 impulses per second ; the relative refractory phase, however, lasts for 3 msec., so that at frequencies greater than about 300 p. sec. all impulses after the first one will be slightly reduced (Fig. 103), and, incidentally, are also conducted along the fibre more slowly. When the frequency of stimulation is less than 300 p. sec., all the impulses are of full size, and this is the case whether the stimulus be strong or weak. In frog's nerve at 20° the maximum frequency is about 500 p. sec.

VOLLEYS AND TRAINS OF IMPULSES. These two terms in frequent usage must now be explained. If a maximal stimulus be applied to a nerve at a given point, impulses are fired off simultaneously from that point along all the fibres. (A weaker stimulus will start off impulses along fewer fibres.) The result is in either case known as a *volley* of impulses, in fanciful comparison with the firing of a volley of rifle shots, the essence of the matter being that the impulses all start off at once, like scratch runners in a race. Actually there are, so to speak, two sets of runners, starting back to back and travelling in opposite directions, because the stimulus to each nerve fibre in its course initiates two such impulses in opposite directions. It will be seen that this is one way in which many impulses may be sent along a stimulated nerve, one along each fibre in each direction. It is clear that this is a sort of *spatial summation*, corresponding to quantal summation in a muscle.

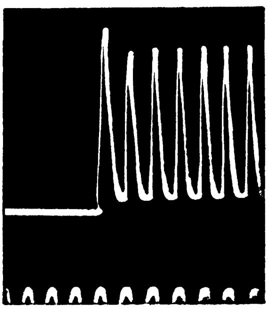

FIG. 103. Stimulation of Phrenic Nerve at 1,075/sec. All but first response are reduced. (GASSER. *J Appl. Physics*, 1938, **9**, 88.)

Another type of summation, which may be called *temporal summation*, exists when more than one stimulus is given to a nerve fibre. In this case, within limits to be described later, each stimulus causes a fresh impulse in each fibre, so forming *trains*, consisting of a number of impulses, equal to the number of stimuli, speeding one after the other along the fibre. This does *not* correspond with wave summation in muscle because the nerve impulses never fuse together, but always remain discrete.

By repetitive stimulation of a nerve both types would be combined, *i.e.* there would be trains of volleys.

REFRACTORY PERIOD. For a short time after its excitation a nerve fibre is in a state of altered excitability. If two 'adequate' stimuli are applied to a frog's sciatic nerve within a sufficiently brief interval, the second stimulus is ineffective. This is the *absolute refractory period.* It is longer the lower the temperature and for frog nerve is about 2 msec. (0·5 msec. to 3·0 msec.) at 10° C.,* the Q10 being 3·0 (*i.e.* the duration being reduced to one-third by a rise of 10° C.). It is lengthened if the excitability of the nerve is depressed by narcotics. Following the absolute refractory period, the nerve remains in a less excitable state than normal for a further short

* AMBERSON. *Journ. Physiol.*, 1930, **69**, 60.

period, during which it may only be excited by stronger stimuli than were needed for the first, or *conditioning*, stimulus, and during which time also the impulses generated are of smaller size. This is the *relative refractory period.*

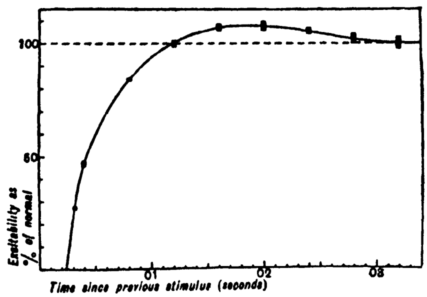

FIG 104. Recovery of Excitability of Frog's Nerve (Adrian and Lucas.)

These periods of diminished excitability immediately after the application of a stimulus are succeeded by one during which the nerve is more excitable,* the *supernormal phase* (Fig. 104), and this again by another phase of *subnormal excitability*. For the large (A) fibres of mammals the absolute refractory period is about 0·4 msec., relative refractory phase 3 msec., supernormal phase 12 msec., and subnormal phase up to 70 msec. from stimulation (Fig. 105).

When the nerve has been submitted to several conditioning stimuli

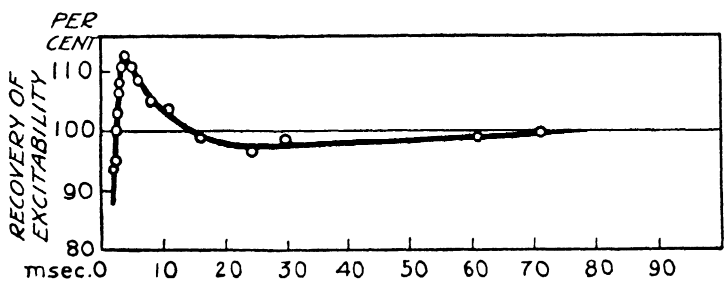

FIG 105 Recovery of Excitability of Cat's Saphenous Nerve stimulated at 0. (GASSER and GRUNDFEST. *Amer J Physiol*, 1936, **117**, 129)

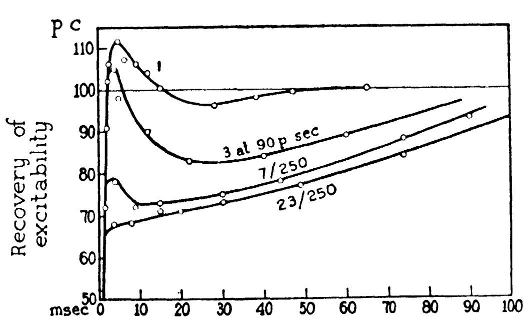

FIG 106 Excitability of saphenous nerve of cat *in situ*. Ordinates: excitability compared with resting threshold Abscissæ intervals between last of the conditioning shocks and testing shock. Numbers over the curves show the number and frequency of the conditioning shocks, *e g*, 7/250 means 7 shocks at the rate of 250 per second. The upper curve is after one conditioning shock only (GASSER and GRUNDFEST. *Amer J Physiol*, 1936, 117)

previous to the testing shock, the picture is somewhat altered, inasmuch as the supernormal phase and the negative after-potential are reduced or missing, the extent of the reduction being in proportion to the frequency and

* ADRIAN and KEITH LUCAS. *Journ. Physiol.*, 1912, **44**, 68.

number of the preceding stimuli. as is shown in Fig. 106. The positive after-potential, and subnormal phase are proportionately increased.

It is an important consequence of the refractory period phenomenon that if two impulses travelling in opposite directions along the same nerve fibre meet one another, each will enter the refractory phase left behind the other, so that both will be extinguished. Another consequence is that a nerve can only carry a limited number of impulses per second. The refractory period is lengthened during continuous transmission at high frequencies.*

Latent Addition. The summation of two *inadequte* stimuli is possible in a nerve, provided these are not more than 0·5 msec. apart, *i.e.* the first effect is to make the nerve more excitable. For several msec. afterwards, however, the nerve is in a relatively refractory state, even though the stimulus was ineffective.† Hence stimulation by repetition of sub-threshold stimuli may never occur if each stimulus falls in the refractory phase of its predecessor; or if the stimuli are adequate in strength, but suitably spaced (*e.g.* frog's nerve stimulated at 1000 p. sec.), only the first one is effective (Wedensky inhibition).

ALL-OR-NONE CHARACTER OF THE IMPULSE. We must regard the nervous impulse as some form of energy transmission, since it is capable on arrival at its destination of throwing its effector organ into activity. A very important question is that concerning the type of propagation. Is it transmitted passively along the conducting medium like sound, or does the nerve fibre contribute energy which keeps the impulse alive and transmits it from point to point, as, for example, in the travelling of combustion along a train of gunpowder ?

In the former case the impulse must get progressively weaker as it travels along, and its damping-down could, under certain conditions, be greatly accelerated, as, for example, that of sound in encountering a layer of cotton wool; in other words, the nervous impulse would normally suffer a *decrement* from which, in its further passage along its course, it would not recover.

If the latter case were the correct one, the impulse would maintain a constant (all-or-none) intensity so long as the conditions were constant and, although its progress might be damped down locally, as in a stretch of burning fuse which had been moistened, it would, if it succeeded in emerging from such a region of depression, again flare up to its normal level.

Adrian‡ came to the conclusion that, after having passed through a region of impaired conduction brought about by local narcosis, the nerve impulse did again spring up to its former level on emerging into a normal stretch of nerve, and subsequent work has thoroughly substantiated this conclusion. That the nerve impulse normally suffers no decrement is clearly shown by the fact that the action potential as it passes along a normal nerve fibre remains of the same size. If a part of the nerve is narcotised, the action potentials are reduced in magnitude in that narcotised stretch of nerve, but if not completely quenched become normal again in the non-narcotised portions beyond.§

Adrian|| has pointed out that the all-or-none response of nerve is a logical consequence of its having a threshold of excitability and a refractory period. A stimulus in order to excite must produce a certain minimal local change, and the propagated disturbance which is set up as soon as this change has

* Bugnard and Hill. *J, Physiol.*, 1935, **83**, 394.
† Erlanger and Blair. *Amer. J. Physiol.*, 1931, **99**, 108.
‡ Adrian. *Journ. Physiol.*, 1912, **45**, 413.
§ Davis *et al.* *Amer. Journ. Physiol.*, 1926, **76**, 448.
|| Adrian. *Loc. cit.*

reached its critical value, must therefore be of an all-or-none character. A stimulus beyond threshold strength 'may cause the local change to exceed the value required for excitation, but the disturbance will have been set up at the moment when the critical value was reached, and the refractory state accompanying the disturbance will prevent any subsequent change in the local conditions from affecting its size in any way.'

THE EFFECT OF TEMPERATURE ON EXCITABILITY. The threshold of excitability of a nerve is within certain limits lowered by cooling the nerve and increased by raising its temperature. Thus if a frog be cooled to 2° C. or 3° C. for a day, it will be found that section of the sciatic nerve may send the gastrocnemius into continued contraction, and under these circumstances 'closing tetanus' may be obtained with the greatest ease. This increase of 'excitability' does not, however, imply a diminished chronaxie, but only a reduced rheobase. Actually the chronaxie of both nerve and muscle are increased by cooling, and therefore they are *less* excitable for induction shocks and for galvanic currents of short duration.

When a nerve trunk is warmed to a certain temperature (32° to 40° C. for various species of frogs) it becomes temporarily inexcitable (heat paralysis), but the excitability is restored again by cooling.*

The excitability and conductivity of nerve are both abolished below a certain temperature, *e.g.*, 5° C. in the mammal.

THE EFFECT OF INJURY. The excitability of the nerve of a nerve-muscle preparation is not equal in all parts of its course, but is greater at the upper end, probably because it is nearer to a site of injury.

Some time after a motor nerve is divided, the increased excitability at the upper end gives way to a decreased excitability, and this decrease goes on till the nerve is no longer excitable. The diminution in excitability gradually extends down the nerve fibre, so that the part of the nerve nearest the muscle remains excitable the longest (the Ritter-Valli law). It is soon followed by definite histological changes in the nerve.

PROPAGATION ALONG NERVE FIBRES

Although nerve fibres have the power of conduction in both directions *the impulse is normally only conducted in one direction*, starting at one end of the fibre and travelling to the other end. In consequence we find in the body a clear differentiation of function between various nerve fibres. Thus Bell and Magendie showed that the ventral spinal roots carry only impulses from spinal cord to periphery, while the dorsal roots carry impulses from periphery to central nervous system. The law known by the name of these observers states, indeed, that a nerve fibre cannot be both motor and sensory. We often find both kinds of fibres contained in a single nerve trunk, but normally each fibre conducts impulses only in one or other direction. (The 'axon reflex,' referred to later is an exception to this, which need not be considered here.) Under normal conditions the afferent fibres are excited only at their specialised peripheral endings, while the efferent fibres are excited only at their origin within the spinal cord. The difference in the function of different nerve fibres depends therefore not on the structure of the nerve fibre itself, nor on differences in the impulses conveyed, but on the different connections of the fibres. We can show this experimentally by grafting one set of nerve fibres on to another.† If the cervical sympathetic be united to the phrenic nerve, stimulation of the sympathetic, instead of causing, as usual, constriction of the vessels of the head and neck, will cause contraction of the diaphragm. In the same way the finer functional differences between the various forms of sensory nerves seem to be determined

* THÖRNER. *Z. f. allg. Physiol*, 1920, **18**, 226.
† LANGLEY and ANDERSON. *J. Physiol*, 1904, **31**, 365

by their connections within the central nervous system. Stimulation of the optic nerve by any means whatsoever evokes a sensation of light. One and the same stimulus applied to different nerves will evoke different sensations, *e.g.* a tuning-fork applied to the skin will give a sensation of vibration, to the ear a sensation of sound.

The velocity of propagation along a nerve was first measured by Helmholtz.* In a simple modification of his method, a frog's gastrocnemius is prepared, with a long piece of sciatic nerve attached. The muscle is arranged (Fig. 107) so that its contraction may be recorded on a rapidly moving surface, on which are also shown the moment at which the stimulus is sent into the nerve, and a time tracing. Records are now taken of the contraction of the muscle when the nerve is stimulated: first, at its extreme upper end; secondly, as close as possible to the muscle. It will be found that the latent period, between the point at which the stimulus is sent into the nerve and the point at which the lever begins to rise, is longer in the first case than in the second. The difference in the two latent periods gives the time that

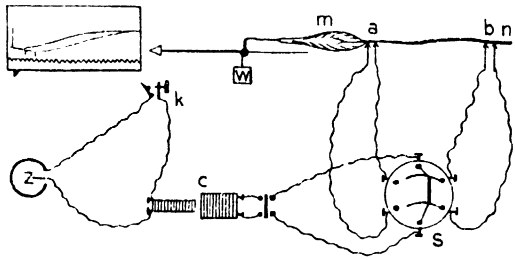

FIG. 107. Diagram of Arrangement of Experiment for the Determination of the Velocity of Transmission of a Motor Impulse down a Nerve.

The battery current passes through the primary coil of the inductorium *c*, and a 'kick over' key *k*. By means of the switch *s*, the break shock in the secondary circuit can be sent through the nerve *n*, either at *b* or at *a*. The muscle *m* is arranged to write on the blackened surface, and is excited during the passage of the recording surface by the automatic opening of the key *k*. (The time marker is not shown.)

the nervous impulse has taken to travel down the length of nerve between the two stimulated points. In this way, the velocity of propagation in frog's nerve is found to be about 30 metres per second. In man and in warm-blooded animals the velocity is about 120 metres per second.†

These values represent the *highest average* velocities in motor nerves. As a matter of fact, the impulses generated in a nerve of mixed function travel at different speeds, the coarse fibres conducting most quickly.‡

There is a more refined method of determining the velocity of conduction, which may be used also with sensory fibres. This is by recording the times at which the monophasic potential spike passes two points at a known distance apart along the nerve.§ We have seen that if a nerve is stimulated a volley of impulses is started simultaneously in many fibres. But, like runners starting from scratch, the further they go the more the fast ones will outstrip the others. By taking records at various distances Erlanger and Gasser found that the potentials, at first all so close together as to appear homogeneous, separated out on further travelling into groups of different speeds,

* HELMHOLTZ. *Arch. (Anat. u), Physiol.*, 1850, 71; 1852, 199.
† PIPER. *Pfluger's Arch.*, 1908, **124**, 591.
‡ ERLANGER. *Harvey Lecture Series*, **22**, 1926–27, 90.
§ SCHMITT. *J. Physiol.*, 1938, **94**, 21P.

the slower groups appearing as new waves on the descending limb of the composite potential record, as illustrated in Figs. 108 and 109.

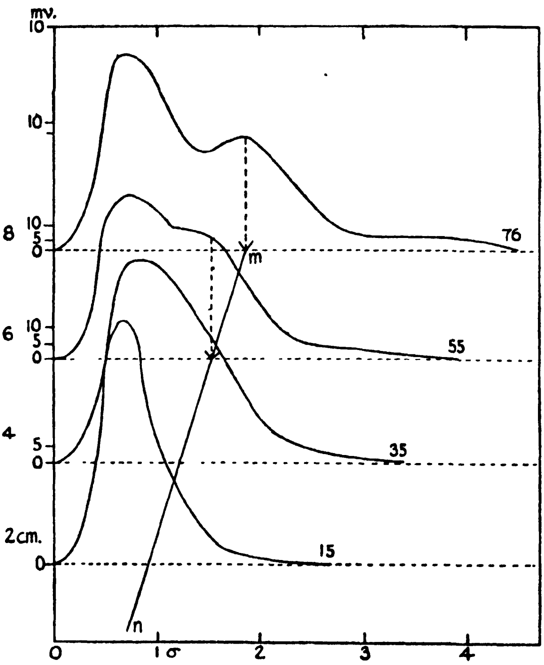

FIG. 108. Separation of Nerve Action Potentials of Frog Nerve into Components of different speeds by leading off at different distances from their origin. The figures on each curve shows distance. (GASSER, ERLANGER and BISHOP.)

Erlanger and Gasser have divided the mammalian fibres as follows :—

Group A are somatic medullated fibres, motor and sensory. The motor fibres are large, and, together with the largest sensory fibres, are called

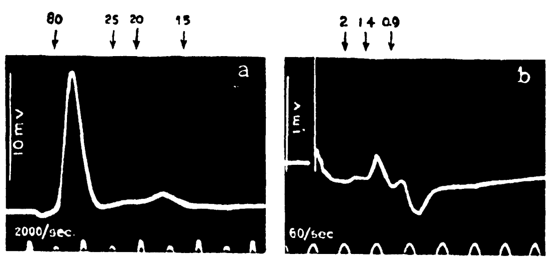

FIG. 109. Saphenous Nerve of Cat. Separation of action potentials after 4 cm. of conduction. On the left the record is on a faster 'surface' than on the right and is less amplified. The spike of the left-hand figure is only faintly seen as a line in the right-hand one. The slowly recorded right-hand figure shows that the slow spikes start from a lowered line—the positive after-potential. The figures above indicate position of impulses which travel at the speeds shown (mm. p. sec) (GASSER. *J. Appl. Physics.*, 1938, **9**, 88.)

α-fibres; these have the shortest chronaxie and the most rapid propagation rate—up to 120 m.p.sec. The smaller sensory fibres, β and γ, have longer chronaxies, and conduction rates down to 30 m.p.sec. ; their functions are separate.

Group B consists of autonomic medullated fibres, of fine calibre, longer chronaxie, and with conduction rates from 11 to 17 m.p.sec.

Group C consists of fine non-medullated fibres. These only conduct at 0·3 to 1·6 m.p.sec.

INTERACTION BETWEEN ADJACENT NERVE FIBRES. We have seen in Chapter X that a nerve may easily be stimulated by the action potential of a muscle, or of another nerve, placed in contact with it. This being so, the question may well be asked whether the fibres in a nerve trunk would not similarly be affected when adjacent fibres were conducting impulses. The answer seems to be * that they are affected, inasmuch as they show excitability changes, but that the effect is not enough to produce actual excitation. There is also an effect when impulses travel simultaneously along fibres lying side by side, whereby the conduction rate of a lagging impulse is speeded up until it catches up with the other, the two thereafter keeping pace. These facts apply to non-medullated nerves and are possibly less conspicuous in medullated ones.

CHEMICAL AND THERMAL CHANGES IN THE NERVE DURING TRANSMISSION. In nerve, which serves merely as a conducting medium, there is less expenditure of energy on excitation than in muscle. All that is necessary is that each section of the nerve should transmit to the next section just so much energy as is needed to excite it.

That the excitatory process in nerve probably produces small chemical changes is indicated by the facts that, in the complete absence of oxygen, the nerve fibres lose their excitability, and that this loss of excitability is hastened by repeated stimulation of the nerve. When the excitability has been abolished by stimulation in the absence of oxygen, it may be restored within a few minutes by re-admission of oxygen to the nerve, and still more quickly if the nerve be also washed in Ringer's solution.

Nerve continually uses oxygen and evolves carbon dioxide; in the absence of oxygen, lactic acid accumulates, as it does in muscle, and on re-admission of oxygen, disappears again, with increased oxygen usage.† When the nerve is stimulated, the rate of oxygen usage is accelerated.‡

Observations of heat production of nerve by A. V. Hill and his pupils also give definite information with regard to the energy exchanges. Frog's nerve at rest evolves about 4×10^{-3} cal./g./min. when in oxygen, and about one-quarter of that amount when in nitrogen.§ If returned to oxygen after being in nitrogen, the heat production is increased.

When impulses pass along the nerve, the heat production is increased, and three phases may be recognized,‖ the first being called 'initial' heat and the other two 'recovery' heat. Initial heat is small in amount, *e.g.* 5 to 10 micro-calories (*i.e.* 5–10×10^{-6} cal.) per sec. per g. nerve during continuous rapid excitation. It dies away at once when stimulation ceases. The delayed heat of recovery consists of an early rapid phase, about equal to the initial heat, and a prolonged phase for 30 minutes or more, which totals about thirty times the initial heat.

The heat produced by a single volley of impulses is of course much smaller, viz. about 0·067 micro-calories per g. of frog nerve at 20° C.

An explanation of these various facts is as follows: ¶ The nervous impulse is a wave of depolarization which rapidly moves over the polarized

* KATZ and SCHMITT. *J. Physiol.*, 1940, **97**, 471.
† GERARD and MEYERHOF. *Bioch Ztschr.*, 1927, **191**, 125.
‡ GERARD. *Amer. J. Physiol*, 1927, **82**, 381; FENN. *J. Gen. Phys.*, 1927, **10**, 767.
§ BERESINA. *J. Physiol*, 1932, **76**, 170
‖ HILL. *Proc. Roy. Soc.*, 1933, **113B**, 345.
¶ HILL. *Proc. Roy. Soc*, 1929, **150B**, 153.

membranes at the surfaces of the fibres, thus also causing the action potential. The depolarization means that ions previously held in position on the polarized membrane are now free to wander. This change would involve but little liberation of energy and so necessitate little if any chemical change or heat production. Hence it occurs equally well at first whether oxygen is present or not. But the process of *recovery* involves repolarizing the membranes, which means that energy must be expended, oxygen used and heat set free, in order to separate the oppositely charged ions and set them in order again. This explains why these various changes are associated with the recovery of the nerve rather than with its actual functioning, and also why the excitability is lost when the nerve is stimulated repeatedly in absence of oxygen, and restored by its re-admission.

The hypothesis also explains another phenomenon called the 'retention of action current' which is well seen in cold-blooded non-medullated nerves.* When the nerve is repeatedly stimulated, especially in absence of oxygen, the action current subsides more and more slowly, or, in other words, the depolarized state persists, as a negative after-potential. If oxygen is admitted the current subsides more quickly again, because the energy necessary to repolarize the membranes is obtainable. The more rapidly acting medullated nerves of the higher animals do not show the phenomenon so clearly, but it is demonstrable in them too. The refractory period is, indeed, due to the fact that repolarization takes an appreciable time.

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CONDITIONS AFFECTING THE PASSAGE OF A NERVOUS IMPULSE

TEMPERATURE. Below a certain temperature the propagation of the excitatory process in the nerve is abolished. In the frog it is necessary to cool the nerve below 0° C. before conduction is abolished, whereas in the mammal it is sufficient to cool the nerve to somewhere between 0° and 5° C. Since cooling the nerve does not excite it, this procedure forms a convenient method for blocking the passage of impulses along a nerve without using the irritating procedure of section. On again warming the nerve the conductivity returns. The rapidity with which the excitatory process is propagated along nerve fibre depends on the temperature. Thus, the mean rate of conduction in the frog's nerve at 8° to 9° C. is only 16 metres per second. The temperature coefficient of the velocity of nerve propagation, *i.e.* $\frac{\text{velocity at T} + 10^\circ}{\text{velocity at T}^\circ}$, is about 1·58 between 12° and 26°, but below 12° is higher.†

THE INFLUENCE OF REPEATED STIMULATION. Medullated nerves are not

* FURUSAWA. *J. Physiol.*, 1929, **67,** 325.
† ROSENBERG and SUGIMOTO. *Bioch. Ztschr.*, 1925, **156,** 262.

at all easily fatigued, in fact they are almost indefatigable unless deprived of oxygen. This may be shown in various ways. Thus, a nerve can be isolated and repeatedly stimulated, say, at 100 per second, while its action potentials are observed on a cathode ray tube ; they will be found to continue unchanged for long periods. It may be demonstrated in mammals by poisoning the animal with curare, and then stimulating a motor nerve continuously while the animal is kept alive by means of artificial respiration. As the effect of the curare begins to wear off in consequence of its excretion, the muscles supplied by the stimulated nerve enter into tetanus. The action of the curare may be cut short at any time by the injection of eserine, when the muscles will at once begin to react to the excitation.

The same fact may be shown in the frog. The gastrocnemii of the two sides with the sciatic nerves are dissected out, and an exciting circuit is so arranged that the interrupted secondary currents pass through the upper

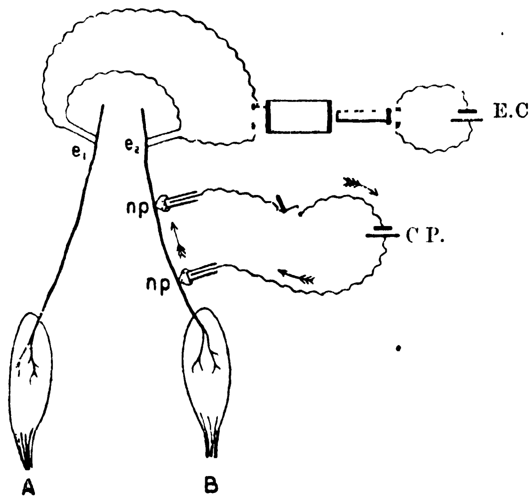

FIG. 110. Arrangement of Experiment for demonstrating the Relative Absence of Fatigue in Medullated Nerve Fibres.
E C, exciting circuit ; C P, polarizing circuit.

ends of both nerves in series (Fig. 110). At the same time a galvanic cell is connected with two non-polarizable electrodes (*np*, *np*) placed on the nerve of B, so that a current runs in the nerve in an ascending direction. The effect of passing a constant current through a nerve is to block the passage of impulses beneath the anode. The exciting current is then sent through both nerves by the electrodes e_1 and e_2. The muscle A enters into tetanus, which gradually subsides owing to 'fatigue.' When A no longer responds to the stimulation, the constant current through the nerve of B is broken. B at once enters into tetanus, which lasts as long as the contraction did in the case of A, and gradually subsides as fatigue comes on. Since both nerves have been excited continuously, it is evident that the fatigue does not affect the nerve trunk.

But although repeated stimulation does not reduce the nerve to an inexcitable and non-conducting state it would be wrong to suppose that it produces no change at all. The phenomena of the refractory period and the after-potentials are indications of incomplete recovery, and hence if stimuli follow one another with sufficient rapidity, certain consequences follow. If the intervals between stimuli is shorter than the relative refractory period, the

second and subsequent stimuli produce smaller spikes than the first one (*v.* Fig. 103), and may show a progressive decline. Also as stimulation continues the negative and positive after-potentials increase, and during the positive after-potential the excitability and conduction velocity are subnormal.*

Since a muscle stimulated to fatigue *via* its nerve will respond to direct stimulation we must therefore conclude that the phenomena of fatigue are here due to failure of the excitatory process to be transmitted from the nerve to the muscle.

In the normal intact animal the break in the neuro-muscular chain, which is the expression of fatigue, occurs still higher up, *i.e.* in the central nervous system, and is probably due to some reflex inhibition, from the muscle itself, of the central motor apparatus.

CHEMICAL INFLUENCES. The most important drugs with an influence on nerve fibres are the narcotics. Of these we may mention carbon dioxide, ether, chloroform, alcohol and novocaine.

The action of gases and vapours on the excitability and conductivity of a nerve may be studied by means of the simple apparatus represented in Fig. 111.† The nerve of a nerve muscle preparation is passed through a glass tube which is made air-tight by plugs of normal saline clay surrounding the nerve at the two ends of the tube. By means of two lateral tubulures, a current of CO_2 or air charged with vapour of ether or other narcotic can be passed through the tube. The nerve is armed with two pairs of electrodes which are stimulated alternately, the pair within the tube serving to test the action of the drug on the *excitability*, while the pair outside the tube show the presence or absence of any effect on the *conducting power* of the nerve below it.

The substances mentioned above diminish, and finally abolish, the excitability response and conductivity of the nerve fibres. On removing the narcotic, recovery occurs if the dose has not been too great. It has been shown that the nervous impulses (action potentials) are reduced in magnitude while passing through the narcotised stretch of nerve, but regain their normal size on emerging into untreated tissue.‡ If the narcosis is severe the impulses are extinguished altogether so long as it lasts. Advantage is taken of this fact for causing a temporary loss of function in nerve trunks for clinical purposes. Thus, if novocaine is applied to a nerve trunk, all painful sensations from the part supplied by the nerve are blocked, and operations may thus be performed under "local" or "regional" anæsthesia. If applied to the spinal cord (by lumbar puncture), extensive operations on the abdomen or legs can be carried out painlessly.

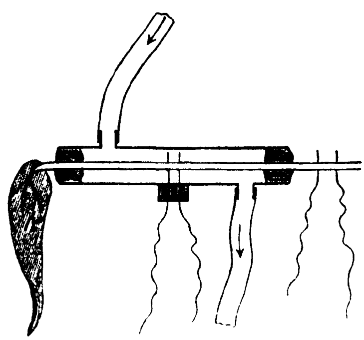

FIG. 111.

Changes in hydrogen-ion concentration do not affect the spikes, but alter the excitability and the after-potentials. Increase of *c*H reduces excitability and reduction of *c*H increases it, until at *p*H 8·0 spontaneous discharges occur.§

Removal of calcium produces the same effects as reduction of *c*H, which is, in fact,

* GASSER. *Amer. J. Physiol.*, 1935, **111**, 35.

† GRUNHAGEN. *Pfluger's Arch.*, 1872, **6**, 180.

‡ ADRIAN. *J. Physiol.*, 1913, **45**, 389 ; DAVIS, FORBES, BRUNSWICK and HOPKINS. *Amer. J. Physiol.*, 1926, **76**, 448.

§ LEHMANN. *Amer. J. Physiol.*, 1937, **118**, 600.

probably due to de-ionsation of calcium. Removal of potassium produced the opposite effects.*

Asphyxia reduces the size of the response, *i.e.* of the spike and after potentials, and also reduces excitability.†

ELECTROTONUS AND POLARIZATION PHENOMENA

ELECTROTONUS. When a constant current is passed along a motor nerve, its direction is called *descending* when the anode or positive pole is the further electrode from the muscle, and ascending when it is the nearer. Such a constant current is often called a *polarizing current*, and many of the phenomena produced by it are due to changes of the state of polarization of certain structures in the nerve. If the nerve be in a very excitable condition, the muscle may be thrown into a tetanus during the whole time that a *strong descending* current is passing ('closing tetanus'). On the other hand, if a strong *ascending* current be passed through the nerve for a considerable time, the muscle when the current is broken may go into 'opening tetanus' for several seconds. Normally, however, the muscle simply responds with a single twitch at the make and break of the current, although, on investigating the condition of the nerve *during* the passage of the current, we find that it is considerably modified. This modification in the condition of the nerve is spoken of as *electrotonus*, and includes changes in its excitability, conductivity and electrical condition.

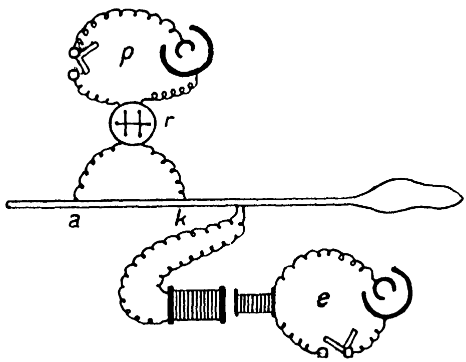

FIG. 112. Arrangement of Apparatus for showing Electrotonic Changes in Excitability.
e, exciting current; *p*, polarizing current; *r*, Pohl's reverser.

To demonstrate the changes in *excitability* the apparatus shown in Fig. 112 may be used, with a nerve muscle preparation. A constant current from the battery is led through a part of the nerve by means of non-polarizable electrodes, about one inch apart. In this circuit we put a reverser and a key. This is the polarizing circuit. The coil is arranged so that we may use break induction shocks, which are applied to the nerve to test its excitability, in the neighbourhood of the pole nearest to the muscle.

We employ submaximal induction shocks throughout the experiment. We now throw a descending polarizing current into the nerve, *i.e.* the cathode is nearer to the muscle. At the make of this current the muscle will respond with a twitch which we may ignore. We now, with the current still flowing, test the excitability of the nerve, and we find that in the region of the cathode the stimulus, which before was submaximal, has now become maximal, as shown by the increase in the height of the contraction evoked by the induction shock.

We now reverse the direction of the polarizing current, so that the current in the nerve is ascending. With this reversal of current there is also a reversal of the changes in the nerve: that is to say, the stimulus when applied near the anode has little or no effect (Fig. 113).

If the testing stimulus be applied between the anode and cathode, we obtain information as to the *conductivity* of the nerve as a result of electrotonus, since the impulses generated have to pass a region of anelectrotonus or catelectrotonus before reaching the

* LEHMANN. *Ibid.*, p. 613.
† LEHMANN. *Ibid.*, 1937, **119**, 111.

muscle. We thus find that in anelectrotonus the impulse may be completely blocked if the polarizing current is a strong one (anelectrotonic block).

On break of the polarizing current the condition of the nerve returns to normal, and the stimulus is once more submaximal throughout. This return to normal conditions is not immediate, since the first effect of breaking the current is a swing back, so to speak, past the normal, the diminished excitability at the anode giving place to an increased excitability which only gradually subsides. In the same way, immediately after the polarizing current has ceased to flow, the neighbourhood of the cathode acquires a condition of diminished excitability, and this only gradually gives place to a normal condition.

This experiment teaches us that, during the passage of a constant current through a nerve, there is an increase in the excitability in the nerve near the cathode, and a diminution in excitability and conductivity near the anode. These conditions of increased and diminished irritability are spoken of as *catelectrotonus* and *anelectrotonus* respectively. Just after the breaking of the current there is a reversal of these states, called the *resolution of catelectrotonus* (or of anelectrotonus). Now the event that takes place at the cathode on make and at the anode on break of a constant current is, as the last experiment shows us, a rise in excitability, in the former case from normal

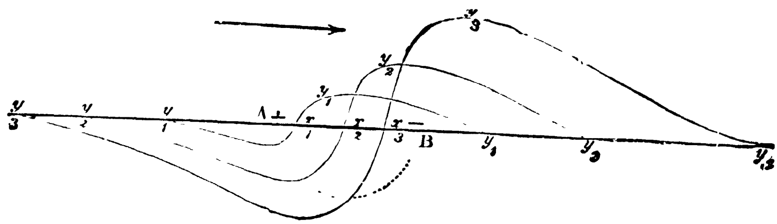

FIG. 113. Diagram to show the Variations of Excitability in a Nerve during the Passage of Polarizing Currents of different Strengths. The degree of change is represented by the distance of the curves from the base line; the part of the curve below the line signifying *decrease*, that above the line *increase* of irritability.

A, anode; B, cathode; y_1, effect of weak current; y_2, medium current; y_3, strong current. It will be noticed that the indifferent point, x, where the curve crosses the horizontal line, approaches nearer and nearer the cathode as the current is increased in strength. (After PFLUGER.)

to above normal, in the latter from subnormal to normal. Hence we may say that the excitation by a constant current is caused by a sudden rise of excitability,* culminating in the discharge of an impulse, which may be due either to a sudden appearance of catelectrotonus or a resolution of anelectrotonus. We have said sudden because the steepness of the change is, as we have seen elsewhere, a necessary factor in causing excitation. The more excitable the tissue, *i.e.* the shorter its chronaxie, the more rapid must the change be if it is to culminate in excitation. In muscle we have seen similarly that a make contraction always starts from the cathode, and a break contraction from the anode.

There must be, somewhere between the anode and cathode, an indifferent point—that is to say, a region where the excitability is neither increased nor diminished. This indifferent point is nearer the anode when the polarizing current is weak; and approaches the cathode as the current is strengthened, so that with very strong currents nearly the whole intrapolar length is in a condition of anelectrotonus (Fig. 113).

When a strong polarizing current is used, the depression of conductivity at the anode is so marked that no impulse can pass this region. Thus if we send a very strong ascending current through the nerve, there is no contraction at make. This is owing to the fact that the impulse started

* ROSENBERG. *Proc. Roy. Soc.* (B.), 1937, **124**, 308.

at the cathode on make of the current cannot reach the muscle, its passage down the nerve being blocked in the region of the anode (Fig. 114, A). Similarly at break of a strong descending current, the excitation originating at the anode is blocked by the great depression at the former cathode (Fig. 114, B).

With the weakest currents excitation occurs only at make, since a

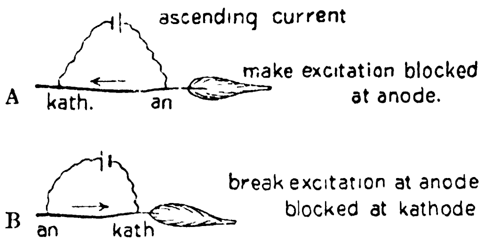

FIG. 114 Diagram to show the Blocking Effect of a strong Constant Current passed through the Nerve of a Nerve-muscle Preparation.

make stimulus, *i.e.* the rise of catelectrotonus, is always more effectual than a break stimulus, *i.e.* the resolution of anelectrotonus.

These results probably hold good for all irritable tissues, and, when tabulated to indicate the results of stimulating nerve-muscle preparations, are sometimes spoken of as "Pflüger's law of contraction."*

If the electrodes are less than 20 mm. apart, the current necessary to

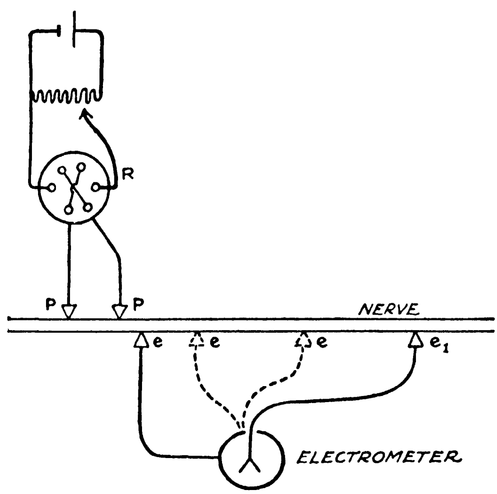

FIG. 115. Measurement of Electrotonic Potentials. (After HECHT.)

excite a nerve is greater the nearer they are to one another. The threshold current leaving the cathode is the same whatever the interpolar length.† But, owing to the greater electrical conductivity of the interstitial fluid as compared with the sheaths of the axis cylinders, the nearer the electrodes are together the greater the fraction of the total currents shunted by the interstitial fluid.

ELECTROTONIC POTENTIALS. If points *e* near one of the electrodes P be tapped off during the passage of a polarizing current, and their potentials

* PFLÜGER. Unters. ü. d. Physiol des Electrotonus. 1859. (Berlin: *Hirschwald*)
† RUSHTON. *J. Physiol.*, 1934, **82**, 332.

compared with that of a more distant point e_1 on the nerve (Fig. 115) by an electrometer, it is seen that the local potentials extend for some distance

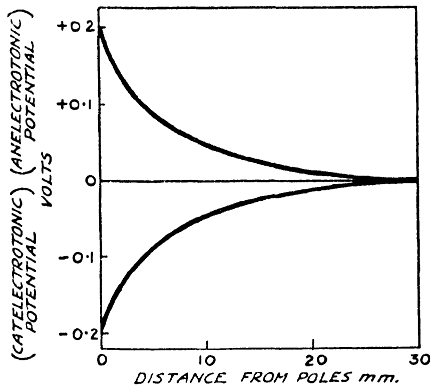

FIG 116. Distribution of Electrotonic Potentials.

beyond the electrodes * (Fig. 116). These are called *polarization potentials*, and are confined to the outer sheaths of the structures concerned.

ELECTROTONIC CURRENTS. If, instead of an electrometer we use a galvanometer

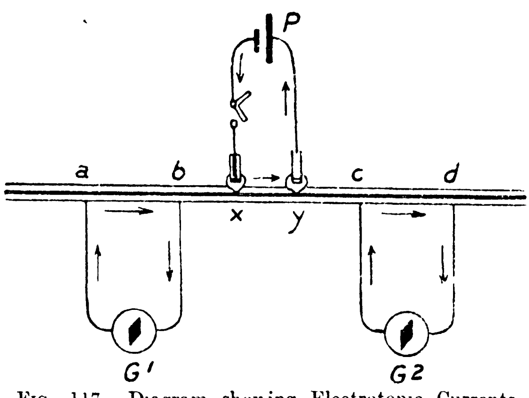

FIG. 117. Diagram showing Electrotonic Currents.
P, polarizing circuit G^1, G^2, galvanometers.

through which a current can pass, between extrapolar points, as illustrated by *a*, *b*, *c*, *d*, in Fig. 117 we find that the needles of both are deflected, and the direction of the deflection shows the existence of a steady current in the extrapolar portions of the nerve from *a* to

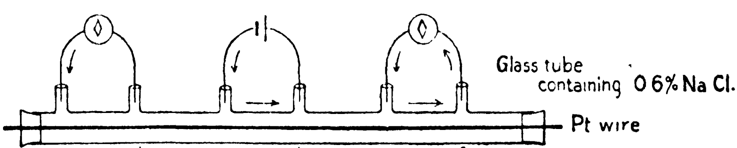

FIG. 118. Apparatus for imitating the Polarization Phenomena in Medullated Nerve ('Core-conductor' model).

b, and from *c* to *d* (Fig. 117), *i.e.* *both* currents are in the *same* direction along the fibre as the polarizing current.

The currents so produced are called the 'electrotonic currents.' They must not be confounded with the action potential which originates at one of the poles only at make

* HECHT. *Z. f. Biol.*, 1931, **91**, 252.

or break of the current, and is transmitted thence along definite fibres as a wave with a measurable velocity. The electrotonic potential, on the other hand, commences almost instantaneously,* takes a very brief time to reach its maximum, then remains nearly stationary, lasts the whole time that the current is flowing through the nerve, and spreads from fibre to fibre. It is caused by the occurrence, or alteration, of polarization in membranes lying between the sheath and the better conducting central part of the nerve fibre, and may be exactly reproduced on a model made up of a glass tube filled with normal salt solution, containing a wire stretched through it (Fig. 118). The polarization in question occurs at the surface of the wire which thus acts as a row of condensers. On leading a current through a and b, and connecting c and d or e and f with a galvanometer, a current will be observed in the extrapolar portion of the model which, as in nerve, is in the same direction as in the intrapolar. A useful model illustrating the electrical properties of nerve may also be made with resistances and leaky condensers.*

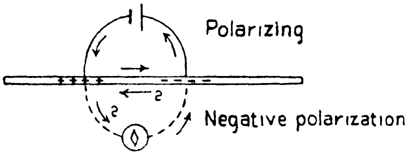

FIG. 119. Diagram to show Direction of the Negative Polarization Current, accompanying a Break Excitation.

In the case of nerve, which normally has polarized membranes, the effect of the constant current is an increase of the natural polarization beneath the anode and a decrease beneath the cathode.

This altered polarization will not disappear at once on breaking the polarizing current. On replacing the battery by a galvanometer, we shall get a current through it in the direction opposite to the previous polarizing current, viz. from anode to cathode (Fig. 119, 2). This is the so-called *negative polarization* of nerve. Similarly in the extrapolar regions of the nerve, we shall have currents in the *same* direction as the previous polarizing and electrotonic currents.

RELATION OF ELECTROTONIC CHANGES TO EXCITATION. When a constant current is led into a nerve the potential at the cathode rises very rapidly from the resting value V_0, by the establishment of an electrotonic potential, V. The rise of electrotonic cathode potential is exponential,† and when it has reached a certain value, say U_0, the extent of the excitation due to polarization change becomes sufficient to be self-propagating. With a current of rheobasic strength this is the fullest polarization the current can produce; if of twice rheobase, excitation occurs when half the possible polarization has occurred, and so on. In other words, for currents of short duration a constant minimal quantity of electricity is needed to produce excitation. This quantity is about 10^{-9} coulombs for medullated nerve, and about 10^{-6} for non-medullated nerves.‡

The time constant k of the change of cathode potential from V_0 to U_0 in the expression:

$$-\frac{dU_0}{dt} = \frac{(U_0 - V_0)}{k}$$

has a value for frog's nerve at 20° of about 0 35 msec., and is related to the chronaxie, which = 0 693 k.§

A second time-factor has been observed by Hill, and is called the *time-factor of accommodation*. It is well known that a slowly rising current has a higher threshold than an abruptly increasing one—the tissue seems to get accustomed to the stimulus and can disregard it until it reaches a greater strength before it responds to it. Let us call the potential at this raised threshold U_x. Then, on passing the rising current the threshold of the nerve has risen from U_0, its former threshold, to U_x at a definite rate, given by the expression:

$$-\frac{dU_x}{dt} = \frac{(U_x - U_0)}{\lambda}$$

where λ is the time-constant of accommodation. Its value is from ten to one hundred times as great as that for k, *i.e.* it is about 35 msec. for frog's nerve: it may vary independently of k, the most remarkable instance of this being produced by alteration in the concentration of Ca ions.|| If the Ca ions are increased the value of λ is greatly reduced,

* BOGUE and ROSENBERG. *J. Physiol*, 1934, **82**, 353.
† BOGUE and ROSENBERG. *J. Physiol.*, 1934, **82**, 353.
‡ ROSENBERG. *J. Physiol.*, 1935, **84**, 50.
§ HILL. *J. Physiol.*, 1935, **83**, 30P.
|| HILL and SOLANDT. *J. Physiol.*, 1935, **84**, 1P.

i.e. the tissue accommodates itself very rapidly, or, in other words, it takes more sudden changes to excite it. Conversely, when the Ca ions are reduced, the tissue becomes hyper-excitable; this is a well known fact in connection with the occurrence of tetany consequent on parathyroidectomy, or over-breathing, which reduce the Ca-ion concentration of the blood.

The accommodation constant may be used to explain many facts in connection with nerve physiology. Thus, with a weak constant or slowly rising current, excitation will not occur until V reaches and passes U_V. Again, the anode cannot excite at make because the anodic V cannot catch up with U, but at break the V returns faster to V_0 than U does to U_0, so that excitation takes place.

Electrotonic changes also occur in other excitable tissues, such as muscle,* but these cannot be considered here.

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ELECTRICAL STIMULI AS APPLIED TO HUMAN NERVES

When we attempt to apply the results gained on frog's nerves to man, we are met at once by the difficulty that we cannot apply stimuli to human nerves directly. So

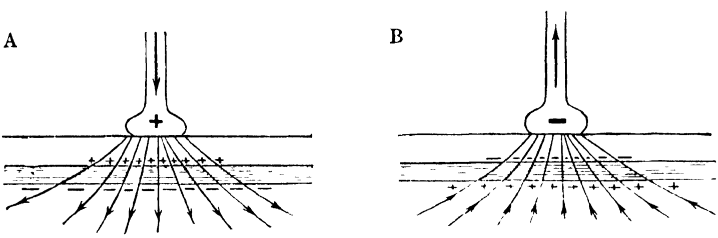

FIG. 120. Electrodes applied to the Skin over a Nerve Trunk.

In A the polar area is anelectrotonic and the peripolar catelectrotonic. The former condition preponderates, since the current here is more concentrated. In B the conditions are reversed, the polar zone corresponding in this case to the cathode. (WALLER.)

usually *unipolar* excitation is used, a small or stigmatic electrode, either anode or cathode, being applied to the skin over the nerve to be stimulated, and the other, a large one, to some indifferent area, such as the back. Under these circumstances the current is concentrated as it leaves the stigmatic electrode, and diffuses widely in the body, seeking the lines of least resistance. Thus it is impossible to get pure anodic or cathodic effects. If the anode be applied over the nerve, the current enters by a series of points (the polar zone), and leaves by a second series (the peripolar zone). The polar zone will thus be in the condition of anelectrotonus, and the peripolar zone in that of catelectrotonus (Fig. 120). The current, however, will be more concentrated at the polar than at the peripolar zone, and so the former effect will predominate. These restrictions in the application of the current cause slight apparent irregularities in the 'law of contraction' as tested on man.

In stimulating the nerves of man for the purpose of determining the conditions of the different muscles, we may use either faradic or galvanic stimulation. The electrodes are covered with chamois leather moistened with salt solution in order to diminish the resistance of the skin. When it is desired to stimulate any given muscle, the stigmatic electrode is brought as nearly as possible over the spot where the muscle receives its motor nerve. These *motor points* have been mapped out, and reference is generally made to a diagram in determining the point for any given muscle. By reversing the

* SCHAEFER and SCHOLMERICH. *Pflüger's Arch.*, 1938, **241**, 310.

current the stimulating electrode may be made either anode or cathode. It is found that stimulation occurs most easily on closure of the current and when the stimulating electrode is the cathode ; with the greatest difficulty when the current is broken and the stimulating electrode is the cathode.* These different contractions are generally represented by capital letters, and in order of increasing difficulty of elicitation we have :—

CCC = cathodal closing contraction.
ACC = anodal closing contraction.
AOC = anodal opening contraction.
COC = cathodal opening contraction.

The problem of the determination of the chronaxies of the human nerves or muscles has been investigated by Bourguignon,† who made the important observation that different muscles (with their nerves) have different chronaxies, and further, that in the same segment of a limb, muscles of similar function have the same chronaxie. Thus when the chronaxie of flexor and extensor muscles is compared it is found that the flexors have a chronaxie only half as great as the extensors. This agrees with the relative duration of the contractions, which Baird and Fulton ‡ find to be about 30 msec. and 40 msec. respectively.

When the motor nerve to a muscle has undergone degeneration, the muscle also begins to degenerate, and we find certain alterations in its chronaxie. For some time after section of the motor nerve the chronaxie of nerve and muscle remains unaltered ; this period may be up to a week or ten days in the sciatic-gastrocnemius of the frog, and at the end of this time the nerve has ceased to function. The chronaxie of the muscle then steadily increases, and at the end of three months is up to five times its normal value. In warm-blooded animals and in man the change takes place much more rapidly.

The muscle after degeneration of its nerve gives a slow contraction and is frequently of such high chronaxie as to be inexcitable to the brief faradic shock (reaction of degeneration). In the second place, qualitative alterations in irritability may be present, so that ACC may be obtained with a smaller current than CCC. Changes in the chronaxie are, however, detectable long before this stage is reached.

THE NATURE OF THE NERVE IMPULSE

Under this heading we have two questions to discuss: (*a*) the nature of the change aroused at the stimulated spot in the fibre, and (*b*) the propagation of the excitatory change away from the stimulated spot and along the fibre.

The all-or-none character of the impulse, together with the normal absence of decrement in the excitatory process, has been taken as an indication that the axis cylinder of the nerve is the seat of energy changes which may be let loose under the influence of chemical or electrical changes, just as the energy of a contracting muscle is set free by the exertion of an infinitesimal force applied as a stimulus.

There is thus some justification for the comparison of a nerve fibre to a train of gunpowder, though in the nerve fibre the impetus to disintegration imparted from each particle to the next in order is only accompanied by a very small rise in temperature, but principally by an electrical change. The excited condition at any segment of a nerve is associated with a development of electromotive forces at the junction of that segment with the adjacent resting segments. The negative pole of a galvanic circuit is the pole at which the stimulus arises. The current of action thereby produced can pass by the sheath of the nerve, so that it must enter the axon at the excited spot, and leave it at the adjacent unexcited segment.§ Hermann suggested that in this way the current of action at any stimulated spot may excite the adjacent

* DE WATTEVILLE. L'electrotonus des nerfs chez l'homme. 1883. (London: De Ranken.)

† BOURGUIGNON. La chronaxie chez l'homme. 1923. (Paris: Masson.)

‡ BAIRD and FULTON. *Amer. J. Physiol.*, 1927, 81, 462.

§ HERMANN. *Hdbch. d. Physiol.*, 1879, II., 194.

segments, causing them to become negative and thus setting up a current of action which in its turn excites the succeeding segments. In this way the excitatory process may travel the whole length of the nerve. Propagation would thus involve the successive setting up of an excitatory process all along the nerve. In more recent years an essentially similar theory has been favoured, and with considerable experimental support, especially by Lillie and A. V. Hill.* It will be readily understood from what has previously been said in Chapter X, and in the present chapter, that according to this conception the cathode stimulates by causing depolarization locally: this leads to the events just described, so that the depolarization (and hence the action current) spreads from point to point along the surface previously polarized (Fig. 121). A drop in the electrical resistance outside the fibre causes a rise in the velocity of propagation of the impulse, as the theory would anticipate.† The depolarized state lasts for a short time at each point, and during this time that point is incapable of stimulation, *i.e.* it is refractory. These changes do not involve any considerable chemical change. It is probable that the surface membrane, separating the axis cylinder from its surroundings, is the site of the propagated disturbance. If we suppose that this is only one molecule thick, it is evident that the amount of energy involved would be very small. If for instance the layer consisted of a

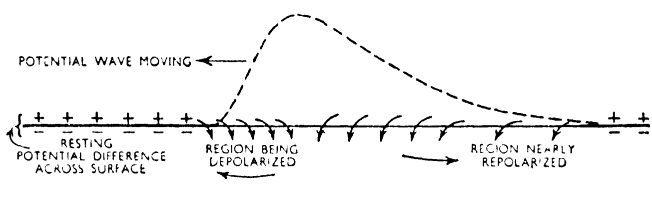

FIG. 121. Diagram to illustrate depolarization theory of nerve impulse. (A. V. HILL.)

monomolecular film of fatty acid on a nerve fibre 10μ in diameter, its volume would be only $\frac{1}{5000}$ of that of the nerve fibre. Thus the active element in a nerve fibre would be a monomolecular film maintained in a polarized state at the expense of the oxidative reactions which normally go on slowly during life. These reactions, and hence the chief energy expenditure, are concerned with restoring the state of polarization after the impulse has passed. Hinder the oxidation and the film remains depolarized, so that the impulse can no longer be transmitted.

The depolarization during activity is accompanied, and perhaps caused, by an increase in the permeability of the nerve membranes. In consequence of this, certain substances, notably potassium, pass out of the fibre into the surroundings. We may suppose that it is this passage of potassium ions through membranes previously able to restrain them from wandering, which constitutes the essential cause of the depolarization. Similar phenomena occur in muscle, and it is perhaps worthy of note that both tissues are readily rendered inexcitable when dilute solutions of potassium salts are applied to their surfaces.

Non-conducted Responses. Since the propagation of the impulse depends on the stimulation of each section of the fibre by the preceding one,‡ it should be possible to apply a localised stimulus of a strength so small that the length of nerve depolarized by it is insufficient to pass on the excitation. Hence we shall expect to get a localised and non-conducted response, and this is in fact what happens.§ At the spot so stimulated,

* A. V. HILL. *Proc. Roy. Soc.* (B). 1936, **119**, 305.

† HODGKIN. *J. Physiol*, 1938, **94**, 560.

‡ HODGKIN. *J. Physiol.*, 1937, **90**, 183, 211.

§ RUSHTON. *Proc. Roy. Soc.* (B), 1937, **124**, 210. KATZ. *Proc. Roy. Soc.* (B), 1937, **124**, 244.

a second stimulus is facilitated by latent addition, and, moreover, the transitory excitation is accompanied by a small and non-propagated action potential. In a block by localised cold, on the other hand, a normal impulse with maximal polarization is transformed down to a subthreshold catelectrotonic charge which is demonstrable on the far side of the block. The explanation is challenged by Blair.*

Lillie's Model. R. Lillie has drawn attention to the striking analogy between the nerve impulse and the change which can be propagated along an iron wire lying 'passive' in nitric acid. If such a wire be immersed in nitric acid of a specific gravity exceeding 1·2, it reacts only momentarily and then enters into a passive state in which no further solution occurs, a thin film of oxide being developed and maintained on its surface. Yet this seemingly stable system is highly reactive. Activation may be brought about by electrical, chemical or mechanical means. For example, if the wire is touched by a piece of zinc, a local electrical circuit is produced and it becomes activated at that spot, and turns black and effervesces ; moreover, the activation thus initiated spreads as a wave of definite velocity over the whole surface of the wire, lasts for a few seconds, and disappears, the iron becoming passive again. But now the freshly passivated iron is resistant to re-activation for a minute or so (refractory period), after which the sensitivity to activation returns again. Mechanical stimulation, summation, electrotonus, polar stimulation on electrical activation, &c., can all be imitated on this fascinating model, as can also some of the facts of the time-relations of electric currents, such as the relative inefficacy of slowly rising currents to activate as compared with currents of abrupt onset. Thus the iron wire in these particular conditions transmits a self-propagating electrochemical change, limited to its surface film and in many respects analogous to the propagated impulse in a nerve.

TRANSMISSION OF EXCITATORY STATE FROM NERVE TO EFFECTOR TISSUE.

The excitatory process travelling down a motor nerve is transmitted to the muscle by the intermediation of the motor end plate. In the end plate the axis cylinder comes to an end, and, after passing beneath the sarcolemma, breaks up into a number of branches (in some cases forming a typical end-arborisation), which are embedded in a small amount of undifferentiated protoplasm containing nuclei (the 'sole plate'). A similar break in structural continuity seems to occur in the central nervous system wherever an impulse is propagated from the axon process of one nerve cell to the body or dendrites of another nerve cell, and the area of contiguity, where an impulse passes from one neuron to another, is spoken of as a *synapse*. The presence of the end-plate between muscle and nerve imposes certain new conditions on the conduction of the excitatory state. One of the most important of these lies in the fact that the conduction across the end-plate, like that across the synapse of the central nervous system, is *irreciprocal*. An excitatory process started in the nerve is transferred easily to the muscle.

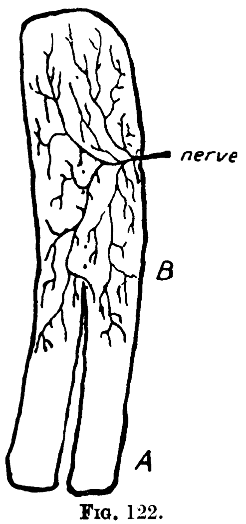

FIG. 122.

* BLAIR. *Amer. J. Physiol.*, 1938, **123**, 455.

On the other hand, an excitatory process started in the muscle does not extend to the nerve fibre. This fact may be shown on the frog's sartorius. If the upper (pelvic) end of the muscle be split, as in Fig. 122, a mechanical stimulus, such as a snip with the scissors, applied to the nerve-free end of one of the limbs, *e.g.* at A, causes a contraction of the corresponding half of the muscle which does not extend to the other half. On snipping the muscle a little further down at B, where nerve endings are present, the resulting contraction involves the whole of the muscle, owing to the fact that the excitation started in the nerve endings spreads in both directions through the branching nerve fibres.

There is evidence that the transmission of the excitatory condition across the end-plate from nerve to muscle involves a special process and the expenditure of energy. If we compare the latent period of a muscle stimulated directly with its latent period when excited through the nerve, we find that there is an increased period of delay in the latter which is not wholly accounted for by the time taken for the impulse to travel from the stimulated nerve point to the muscle. The extra delay is due partly to the fact that the terminal branches of the nerve fibres supplying any motor unit are much finer than the axons from which they spring; they therefore probably conduct more slowly. But there is further delay due to processes occurring in the end-plate. The total delay in passing from the coarser fibres to the muscle is probably about 3 to 4 msec. for the frog's gastrocnemius, but for rapid mammalian (*e.g.*, eye) muscles, it is only 0·7 msec.*

The end-plate seems to be the weakest point in the neuro-muscular chain. We have already seen that, when the nerve of a nerve-muscle preparation is stimulated repeatedly, the muscle very soon shows signs of fatigue, and that the seat of this fatigue is not in the nerve nor in the muscle, but at the junction between the two.

It is probable that the excitation of muscle through nerve depends on the action potential changes occurring at the nerve ending.† If this is so, the discharge must originate in the terminations of the axon and must influence the contractile material of the muscle.

Conduction through a Partial Block. In many respects the conduction of impulses along a partially blocked nerve resembles that across the neuromuscular junction or across a synapse. The properties of a nerve block were first studied from this standpoint by Wedensky.‡ Heat, cold, galvanic polarizing currents, or various chemical agents, *e.g.* cocaine or alcohol have been used to cause the partial block. A partly blocked nerve will conduct impulses at low frequency, but not at high frequency—a phenomenon called Wedensky inhibition. In the blocked region there is a raised threshold of excitation, but beyond the block the threshold is lowered. This difference is accentuated if impulses at a rapid rate are sent into the region and fail to pass through it. When this happens, there is for every impulse arrested at the block a negative variation below the block.§ It is believed that a similar state of affairs obtains at neuromuscular and synaptic junctions.||

Humoral Transmission. There are, however, indications that the excitatory state set up in the muscle may be due to the liberation, at or near the nerve ending, of some potent chemical substance (perhaps acetyl choline)

* LORENTE DE NÓ. *Amer J. Physiol*, 1935, **111**, 272.
† BREMER. *Arch. internat. de Pharmacodynamie et de Therap.* 1930, **38**, 300.
‡ WEDENSKY. *Pflüger's Arch.*, 1903, **100**, 1.
§ HODGKIN. *J Physiol.*, 1937, **90**, 183; 211.
|| LORENTE DE NÓ. *J. Neurophysiol*, 1939, **2**, 402.

which is liberated by the arrival of the nervous impulse, rapidly exerts its action in producing contraction, and then is rapidly destroyed. The denervated muscle, as mentioned on p. 139, responds to a dose of acetyl choline by passing into contracture. If small doses of acetyl choline are injected direct into the arteries to a normal muscle, quick contraction of the muscle results.*

Also, it has been shown that the end-plates contain the enzyme choline esterase in a concentration many thousand times as high as in muscle fibres alone. Hence the acetyl choline could be destroyed with the necessary speed †

It would seem therefore that liberation of acetyl choline at the nerve endings may be a factor in the passing on of excitation from nerve to muscle, and it will be shown later (p. 367) that the transmission of excitation at other points of junction in other tissues is probably also effected by similar chemical transmitters. It is possible, however, that the story does not end here, and that movements of potassium and calcium ions are also concerned, either primarily or secondarily, in the process of transmission.

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* BROWN, DALE and FELDBERG. *J. Physiol*, 1936, **87**, 394.

† MARNAY and NACHMANSOHN. *J. Physiol.*, 1938, **92**, 37.

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THE CENTRAL NERVOUS SYSTEM

CHAPTER XIII

GENERAL FEATURES OF A CENTRAL NERVOUS SYSTEM

THE function of the central nervous system is to enable the individual to respond in a co-ordinated manner to changes in its environment. In the course of its evolutionary development the cells and their processes, the nerve fibres, of which it is essentially composed, have been endowed in an exaggerated form with the properties of excitability and conductivity inherent to lesser degrees in all living protoplasm. Being thus highly excitable and able to conduct the excitatory state with speed and precision for considerable distances, and often to distribute it over wide areas, it is able under appropriate conditions to put remote parts of the body in temporary functional connection with one another and by means of these connections to give close correlation between the actions of such parts.

A nervous system is functionally divisible into three series of parts, the *afferent*, the *central* and the *efferent*. The afferent portions consist of those nervous structures which normally originate and conduct nervous impulses towards the centres, and the efferent portions of those conducting outwards from the centres.

The afferent portions are connected with appropriate sensory receptors and serve to convey nerve impulses aroused by stimuli, *e g.* visual, touch, etc., from the outer world (exteroceptive stimuli), or stimuli arising in the body itself, *e.g.* in viscera or muscles (interoceptive stimuli) to the nerve centres.

The nerve centres receive these impulses, some of which enter consciousness and produce an awareness of the world around us, and of the position and state of our own bodies. It is obvious that our knowledge of the universe, including that of our own bodies, is solely dependent upon, and limited by, these impressions. Many nervous impulses arriving at the centres do not enter the content of consciousness at all, but nevertheless they play an important part in maintaining or regulating the many processes which are constantly proceeding, unknown to us, in the body.

The efferent parts of the system conduct impulses outwards from the centres, and these impulses represent the responses of the centres to the afferent impulses which it has received. When the efferent impulses so generated in, or reflected outwards by, the central portions arrive at their destinations in the so-called *effector organs*, *e.g.* muscle, gland, etc., these are thrown into action and this action represents the response mediated by the central portions as appropriate to meet the particular situation indicated by the afferent stimulus.

The most important stimuli which reach us from the outer world are those of light and sound and to a lesser degree (in man) of smell, since by these we obtain knowledge and warning of things at a distance from us.

Taste, by enabling us to make judgments as to edibility is next in importance. These stimuli are all received by organs placed in the head, which therefore has the prior position in the body, and the sense organs are nearest to the brain which is the main receiving station. Touch is more generalized and requires actual contact with the sensory surface. It is confined to the surface of the body, the deeper structures of which are totally insensitive to touch.

Of the proprioceptive stimuli, important are those that arise in the muscles, joints, tendons, &c. which give us information as to the position of our limbs, &c. those from the labyrinth which tell us of the position of the head in space or of its movement, and those from the various internal organs. Many of the proprioceptive stimuli arouse no conscious sensations.

Two main types of cells go to build up the nervous system, viz. *neurones* and *neuroglia* cells. The latter are supporting cells and are of no immediate physiological interest.

THE NEURONE is the name given to a nerve cell with all its branches. According to the neurone theory, now generally accepted, the neurone is the functional unit of the central nervous system. There are many kinds of neurones, classified variously according to situation, shape, size, number and length of branches, &c., but every neurone consists of two essential parts, viz. first the *soma*, neurocyton or nerve-cell body, and second, its branches, which are nerve fibres (Fig. 123).

The branches of each neurone may vary from one to several in number, but one, and only one, of them is distinguished as the axis cylinder process or *axon*, the others being called dendrites or *dendrons*.

The neurocytons, or nerve cell-bodies, are found in the grey matter of the central nervous axis and in the various ganglia which lie outside it, but not in the white matter, which consists of nerve fibres only. Every nerve fibre, however, is a branch, either an axon or a dendron, of a nerve cell.

The neurocyton, whatever its type, always exhibits certain characteristics of structure on examination by special histological fixing and staining methods.* Although these characters have often been claimed to be produced by the methods employed for fixation and staining, their invariable presence in cells under certain physiological conditions, and their absence or modification in other circumstances, gives them an interest and a claim to further study (Fig. 124). There are, for instance, the Nissl's bodies,† and the Golgi networks, neither of which are visible on ordinary inspection in the living cell-bodies. The Nissl granules are, however, easily seen by photography with ultraviolet light.‡

The Nissl bodies are spindle-shaped masses of basophile material, apparently consisting of a nucleoprotein containing iron. They are of a size, and are arranged in a manner, characteristic for each type of neurone, and are disintegrated in fatigue, by certain poisons, and after section of the axon of the neurone, this process being called *chromatolysis*. Nissl bodies are present in all the hillocks from which dendrites arise, but are absent from the axon-hillock for some unknown reason.

The Golgi networks are of two kinds, an endocellular and a superficial. Their functions are unknown, but following section of the axon the endocellular reticulum is displaced to the periphery of the cell and often fragmented. The fine channels called Holmgren canaliculi are also of unknown function.

The neuro-fibrils seen in the cell-body on special fixation and staining form intricate networks continuous with the neuro-fibrils demonstrable in the axon and dendrons; their properties vary with the physiological state of the cells, and although some consider them to be artefacts, others claim to have demonstrated them in the living cells and fibres.

* GREENFIELD. *J. Neurol & Psych.*, 1938, **1**, 306.
† MOTT. *B.M.J.*, 1912, *ii*, 780.
‡ WEIMANN. *Z. ges. Neurol. Psych.*, 1925, **98**, 347.

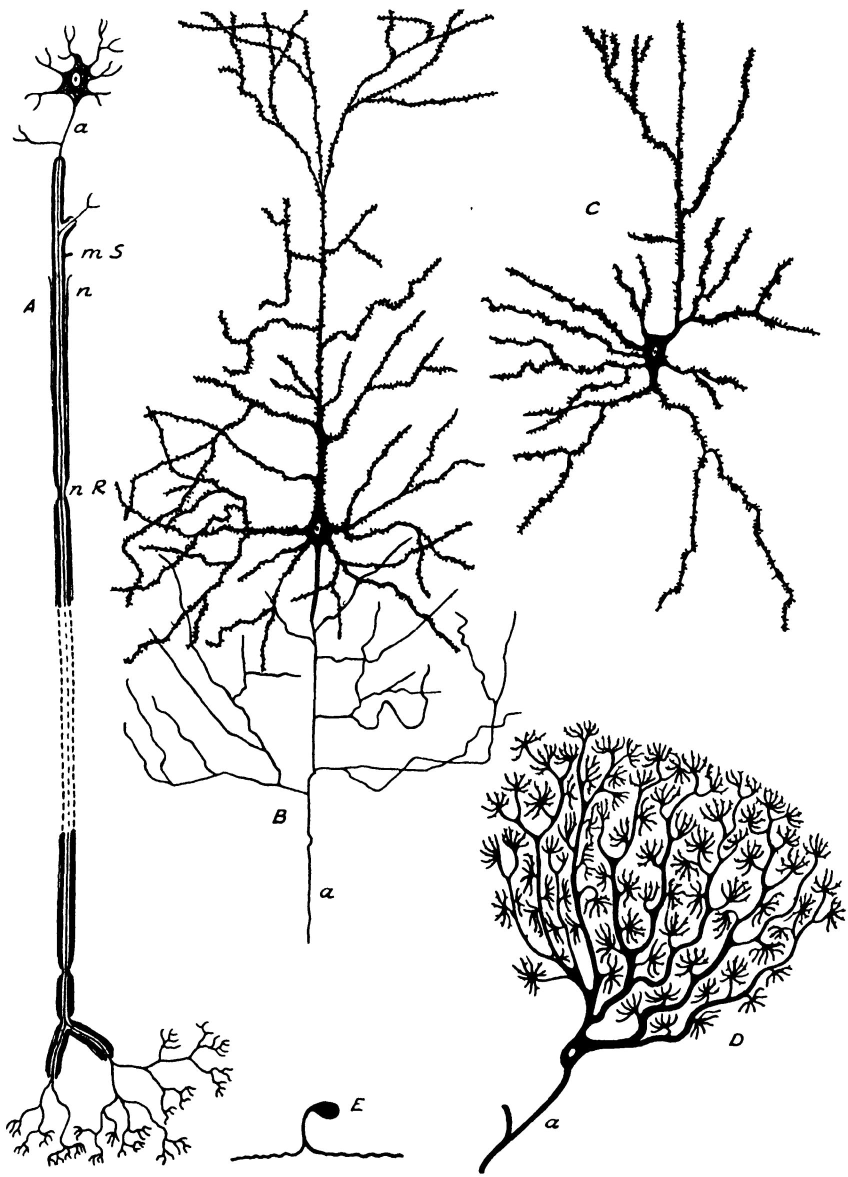

FIG. 123. Characteristic Types of Neurones (diagrammatic).

A Multipolar motor neurone of spinal cord.
B Pyramidal cell of cerebral cortex.
C Golgi Type II cell of cerebral cortex.
D Purkinje cell of cerebellum.
E Bipolar cell of spinal ganglion.
a The axon, is indicated in *A*, *B* and *D*.
n Neurolemma ; *m.S.* medullary sheath ; *n.R.* node of Ranvier.

Each neurocyton has its nucleus, but no centrosome, and it is perhaps for this reason that adult nerve cells are incapable of division. Mitochondria are present in the cytoplasm, where they are visible in the living state.

Many neurones are entirely confined to the grey matter of the central nervous system, and these as a rule have only short axons. They are called Golgi type II. cells. Those neurones that send their axons into the white matter, or into peripheral nerves are called Golgi type I. cells.

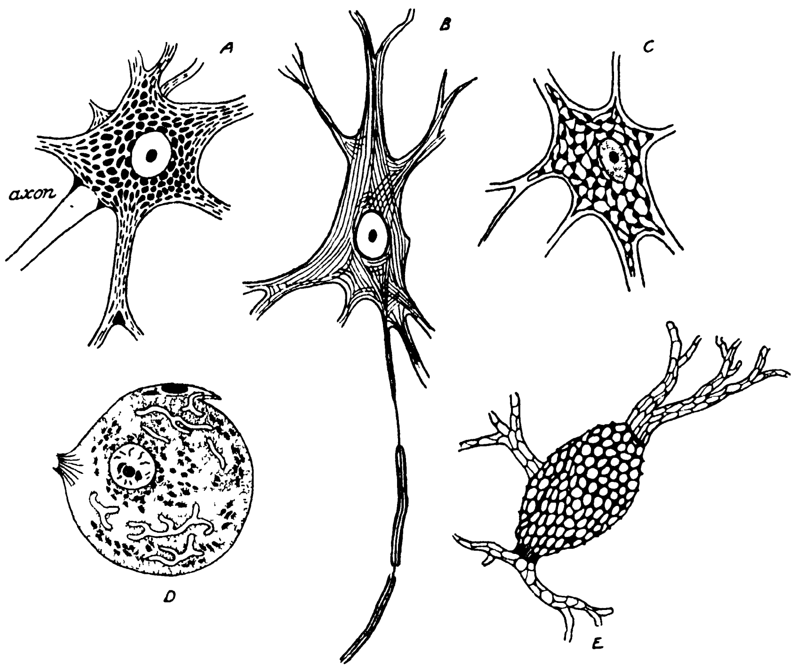

FIG. 124 Illustrating Structure of the Neurocyton

A, Nissl granules in multipolar cell; *B*, neurofibrils in multipolar cell, and addition of medullary sheath to axon; *C*, Golgi endo-reticulum in multipolar cell; *D*, Holmgren's canaliculi, in ganglion cell; *E*, Golgi superficial network in multipolar cell.

The dendrons of a nerve cell, and those parts of its axon which lie within the grey matter of the central nervous system, are composed of neuroplasm and neurofibrils, with no demonstrable cell wall or sheath, but on emerging into the white matter a covering is usually added to the axon, viz. the medullary sheath. To those axons that lie outside the central nervous system a second covering, the neurolemma is added, and in those fibres also the medullary sheath is interrupted at intervals at the nodes of Ranvier.

Terminations of Nerve Fibres. The medullary sheath and the neurolemma are present throughout the peripheral nerves until near their terminations (in efferent fibres) or their origins (in afferent fibres) As these terminations (or origins) are approached the axons branch freely, at nodes of Ranvier, so that several, or many, terminal structures are connected with one axon (Fig. 123A). Motor fibres for instance usually form about 100 branches, each one supplying one muscle fibre. The name *motor unit* is given to the complete structure consisting of motor neurone, with all its branches and all the muscle fibres supplied by them. It functions as a unit of motor activity, In all probability similar arrangements hold good for secretory and other types of neurone. Whatever the mode of termination, it is usual for the medullary

sheath to disappear when it is reached. The neurolemma also sometimes disappears, leaving the terminal nerve fibre as a freely branching net of bare axis cylinder substance. In other instances the neurolemma may fuse with the wall of the cell in which the fibre terminates, as in skeletal muscle, in which the neurolemma and sarcolemma fuse together. The terminal nerve filaments thus come to lie directly in contact with, or may even penetrate into the cytoplasm of the cells at which they terminate, so that the relation between them is of the most intimate nature.

NATURE OF THE CONNECTION BETWEEN NEURONES. The study by His and others of the development of the central nervous system in higher animals showed that from the embryonic cells, the neuroblasts, of the neural groove and crest there are formed by cell division the definite units in the construction of the nervous system which we now know as neurones. These retain their individuality from birth onwards and undergo no further divisions. It is now generally held, at all events for higher animals, that each neurone is a morphologically separate structure having no cytoplasmic continuity with other neurones, and being nutritionally distinct from them.

This conception of the neurone as a discrete structure is largely owing to the Golgi staining method by which certain neurones are stained throughout all their branches, while adjacent neurones are quite untouched. Some observers, however, believe that in invertebrates, and perhaps also in the higher animals, there is definite protoplasmic connection from one neurone to the next by the passage of neurofibrils which bridge the gaps between their processes, *i e* the nerve cells form a syncytium. While it is at present impossible to arrive at a final decision as to the correctness of one or the other opinion, the facts of mammalian physiology seem mainly to be in favour of the neurone hypothesis. According to this hypothesis, connection between one neurone and another adjacent to it can only be effected by the passage of the impulse across some intervening junctional material. This junction is called the *synapse*. At the motor nerve endings in muscle there is also an apparent break of structure at the termination of the nerve in the muscle fibre, any continuity between nerve-ending and contractile substance being subserved by undifferentiated protoplasm. There is thus no difficulty in postulating a similar propagation across a synapse, between the axon of one neurone and the cell body or dendrites of another neurone. If, however, the conception of the evolution of a nervous system from a continuous conducting protoplasmic network be correct, we should expect to find in the fully developed brain and spinal cord some traces of continuity throughout the whole system of neurones. The question as to the existence of anatomical continuity from neurone to neurone has been hotly discussed both for vertebrates and invertebrates. In the case of the latter, evidence in favour of the continuity of neuro-fibrillæ from sensory surface to reacting tissue is strong. The question, however, of the histology of the synapse, presents

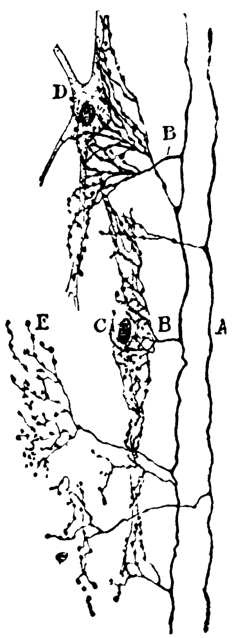

FIG. 125. Arborisation of Collaterals from the Posterior Root Fibres round the Cells of the Posterior Horn. (RAMÓN Y CAJAL.)

much greater difficulties. If we examine a nerve cell such as a cell of Purkinje of the cerebellum, or a cell of Clarke's column in the cord, we find that it is surrounded by a thick basketwork of fibres which are the arborisations or end terminations of the axons which pass to the cell to enter into functional relationships with it (Fig. 125). This pericellular network is of great extent and may equal in total diameter the diameter of the cell itself. Whether the basketwork is really a network, or merely a feltwork in which the fine fibres intertwine among each other without becoming actually continuous at any point, is difficult to make out.

On the periphery of the cell itself another network has been described and is known as the Golgi network. According to Bethe the Golgi network on the one hand receives fibres from the encircling pericellular basketwork of axons, and on the other hand gives off towards the interior of the cell fine fibrils, which are continuous with the neurofibrillæ of the cell and pass out in its axon. There are physiological difficulties in the acceptance of this doctrine of continuity through the central nervous system. Thus in the central nervous system injury to an axon causes degeneration of the portion of the axon below the point of section, but the degeneration stops short at the end arborisation and does not spread into the next neurone. A still more serious difficulty in the acceptance of this theory is the 'Law of Forward Direction,' i.e. the fact that an impulse will pass from an axon to the next neurone, but will not pass backwards across the synapse from the cell body to the contiguous axon. It is possible that we may have to distinguish two types of nervous systems, viz.: (a) A neurofibrillar type, specially developed in invertebrata, with conduction in all directions; and (b) a synaptic type, in which the Law of Forward Direction holds, of later evolution and forming the greater part of the nervous system of vertebrata.

THE SYNAPSE. The synaptic arrangement by which communication can be established between contiguous neurones varies considerably in detail

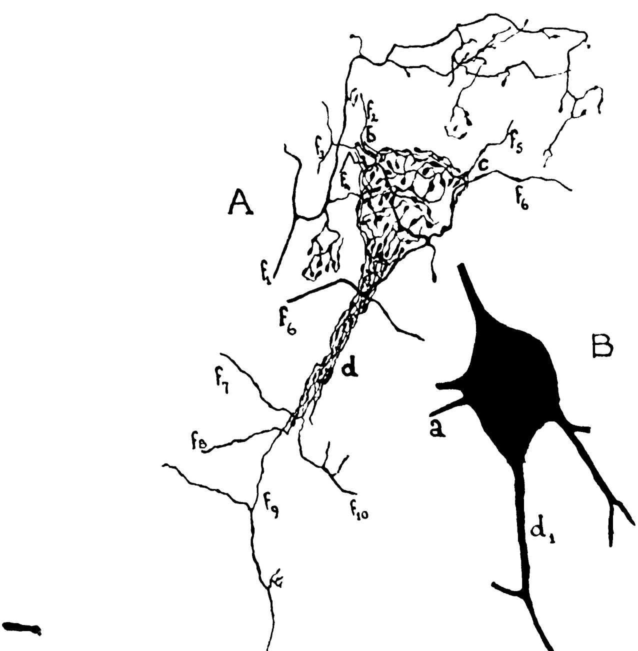

FIG 126. A, synapses on a small internuncial neurone of the ventral ganglion of the acoustic nerve of a 20-day-old cat B, a cell of the same type a, axon; b c,d,d_1, dendrites; f_1 to f_6, fibres having synaptic junctions with cell A. (LORENTE DE NÓ J. Neurophysiol, 1938, 1, 195)

in different situations. In some cases the axon of one neurone and the dendrons of the next both break up into a finely interlaced mass of separate branches, comparable with the interlacing of fine branches of trees growing close together. In other instances there is a plexus or basket of fine axon branches surrounding the body of the cell of the second neurone (Fig. 126). In most types the actual terminals include small buttons or feet (pieds terminaux or better, *synaptic knobs*) applied closely to the cell body or its dendrons (Fig. 127). Despite the variability of these anatomical arrangements there is no reason to suppose that the function of the different types of synapses is dissimilar. In each case there can, under certain conditions, be a physiological connection between the axon of one neurone and the cell-body or dendrons of the next. A nervous impulse can only pass in that direction,

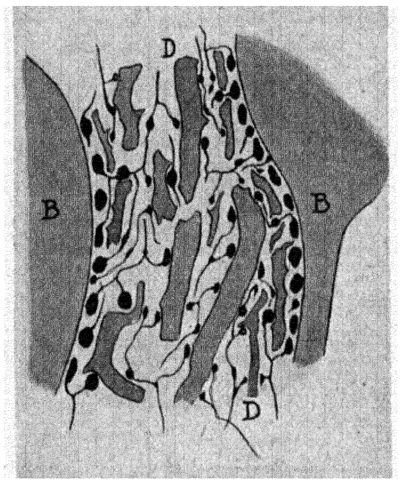

FIG. 127. Diagrammatic Representation of the Articulation of the Dendritic and Fibrillar Plexuses in the Grey Matter of the Spinal Cord. Cell bodies (B) and dendrites (D) in grey; fibrils and synaptic knobs in black. (LORENTE DE NÓ. *J. Neurophysiol.*, 1938, 1, 195.)

from axon to cell body and so on to the axon of the second neurone. At the synapse there is usually a retardation of the nervous impulse, called *synaptic delay*, which we may compare with the end-plate delay occurring on the passage of excitation from muscle to nerve. Not infrequently a synapse is temporarily incapable of transmitting the excitatory state, a condition called *synaptic block*. But under no circumstances is the synapse capable of passing an impulse in the reverse direction from cell or dendron of one neurone to axon of the next, a rule which was called by Sherrington '*the law of forward direction*.' In virtue of this the synapses act like valves allowing impulses to pass in one direction only.

When a stimulus is applied to any part of a neurone, *e.g.* at x in Fig. 128A, it spreads throughout all its parts, and since the normal place for receipt of a stimulus is the dendritic branching or the cell body, the normal direction taken by an impulse will be away from the soma and towards the axon, and along this centrifugally from its origin in the cell body to its terminal branchings, *e.g.* from a to b, whence it will either reach an effector cell or another synapse, which latter, if patent, will be duly traversed, *e.g.* from b to c, so as to pass the excitation on to the next neurone.

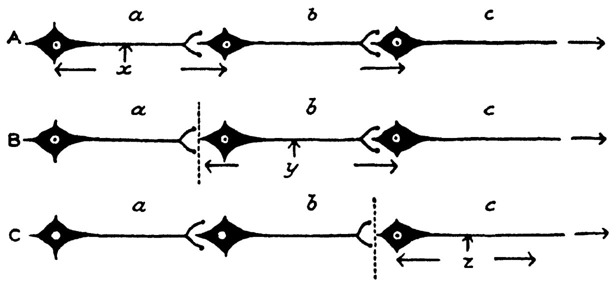

FIG. 128 Diagram to illustrate Forward Conduction.

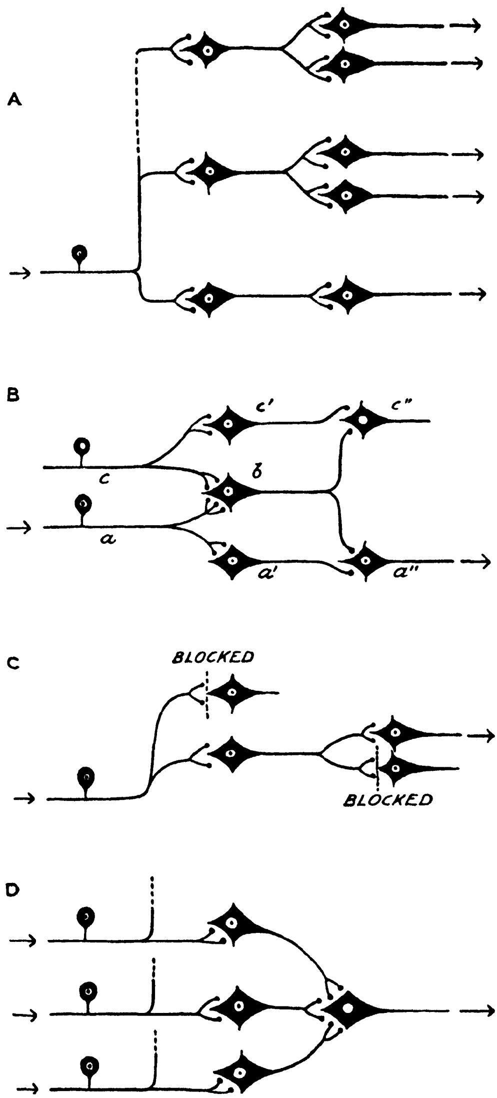

FIG. 129. Diagram to illustrate different connections of neurones.

If by any exceptional means an excitatory state be generated at an abnormal place, *e.g.* in the axon, as at x, y or z in Fig. 128, A, B, C, impulses will travel in both directions from the site of excitation, but only the forward or centrifugal one, *i.e.* which passes in the direction away from the cell body, will succeed in passing the synapse. The centripetal impulse will traverse the cell and dendrons but will be blocked 'up-stream' at the synapse (Fig. 128, B, C).

Distributing, Commutator and Focussing Action of Synapses. From what has been said of the structure of the neurone and of the functions of the synapses, it is evident that the neurones play an essential part in distributing widely the impulses which they receive. This will be readily understood from Fig. 129, A, which shows that impulses received at a single neurone can be dispersed so as to reach many other neurones.

A modification of this dispersing action is that seen in associative connection between neurones, as illustrated in Fig. 129, B. This is frequently of a reciprocative type, *e.g.* an excitation of neurone a may pass on an excitation through neurones a' and b to neurone a'', but the intercalated neurone b may now cause a block at the synapses leading to c'', with the result that as a'' enters into action, any activity in c'' is suppressed. The reverse would hold good for excitations applied to c, the synapse leading to a'' then being in a state of block owing to the action of neurone b.

A further function closely connected with the last is dependent on the fact that not all the synapses may be permeable to impulses, a result of which is that a number of alternative paths are open to impulses passing through a neurone, which thus acts like a commutator or railway junction (Fig 129, C).

Lastly, owing to the multiple interconnections between neurones it is evident that a neurone may function in the opposite way to a distributor and may emit by its axon impulses collected or focussed from widely different sources (Fig. 129, D) to a '*final common path.*' Instances of these three types of action are frequently met with in the central nervous system.

THE MAMMALIAN NERVOUS SYSTEM

It is impossible to give here an account of the anatomy of the central nervous system, for which the reader should consult works on Anatomy and Neurology. All that can be attempted is to recall such outlines as are indispensable to our purpose.

Anatomically, the mammalian central nervous system is a modified tube consisting of the brain, which gives rise to the 12 pairs of cranial nerves, and the spinal cord, from which there emerge 31 pairs of spinal nerves. The hollow portions are modified to form the ventricles and the central canal of the cord.

The brain, or encephalon, is divided, largely on embryological grounds, into the rhombencephalon and the cerebrum, which are united by an isthmus. Of these the rhombencephalon comprises the myelencephalon, or medulla, and the metencephalon, or cerebellum and pons. The cerebrum includes the mid-brain or mesencephalon, the between-brain or diencephalon, and the cerebral hemispheres or telencephalon.

(1) **RHOMBENCEPHALON** (*IVth ventricle*)

(*a*) *Myelencephalon* = Medulla.
(*b*) *Metencephalon* = Cerebellum and Pons.
Isthmus = Superior cerebellar peduncles and superior medullary velum.

(2) **CEREBRUM**

(*a*) *Mesencephalon* = Mid-brain and cerebral peduncles (*Aqueduct of Sylvius*).

Fore-brain:
- (*b*) *Diencephalon* = Hypothalamus, Thalamus, &c. (*Third ventricle*).
- (*c*) *Telencephalon* = Basal ganglia (Corpus striatum, Amygdaloid nucleus and Claustrum)
  Cerebral Hemispheres (*Lateral ventricles*).

The structures remaining after removal of cerebral cortex and cerebellar cortex are sometimes called the Brain Stem.

The spinal cord, and the brain stem and cerebellum, *i.e.* the greater part of the brain apart from the cerebral cortex are chiefly concerned with reflex functions of an instinctive type, and with conduction to and from the cerebral cortex. The cerebral cortex with its appendages is sometimes called the " new brain " (Neëncephalon), and is concerned with the highest functions of association, memory, &c. All its connections with the rest of the body and with the outer world are effected solely through the intermediation of the lower parts, the brain stem, cerebellum and spinal cord.

The details of the anatomical structure of the central nervous system are of little direct interest to the physiologist except in so far as they throw light on function. His attention is directed rather to the attempt to understand the different modes of neural activity, either as studies in themselves, or, more especially in their effects as subserving the co-ordination of functions of the various organs of the body.

The functions performed by each of the parts of the central nervous system will be considered in the chapters which follow. Briefly, we may say that the function of the cord is to act both as a series of reflex centres and also as a conductor to and from higher parts of the nervous system. Histologically, it consists of a column of cells (the grey matter), which is H-shaped in cross-section, surrounded by bundles of medullated axons (the white matter). Of the nerve cells, some give rise to motor axons which leave the cord and go to muscles, some are connected with afferent nerves, to long sensory axons which convey impulses up the cord towards the cerebrum, or to structures below it, *e.g.* to the cerebellum, while some give origin to short-distance axons which correlate the action of neighbouring groups of muscles or subserve reflex acts.

The medulla contains the long path axons which are passing through it on their way to and from the cord, and also various accessory structures. In addition it contains important involuntary 'centres' (control stations) for the heart, blood vessels, and for respiratory movements, as well as the nuclei (relay stations) of many of the cranial nerves. The mid-brain likewise lies on the main track ; and also contains the relay stations of several cranial nerves. The ganglia at the base of the cerebrum act as sorting and relaying stations for impulses which are on their way to the cerebrum. They also have certain specific functions assigned to them which will be described later. The cerebellum lies off the main track, and receives impulses both from the cord and medulla below and from the cerebrum above. It presides over the co-operation of muscles for the performance of skilled acts.

THE BRAIN STEM. It is usual in treating of the structure of the brain stem to consider it as a prolongation forwards of the spinal cord and as consisting, like this, of a central tube of grey matter surrounded by a

tube of white matter. But at the fore end of the body have been developed the organs of special sense, which are the most important in determining the reactions of the animal in response to present or approaching changes in its environment. Hence we cannot expect to find in the brain stem the regularity of arrangement of grey and white matter which is met with in the cord, and the typical division of the grey matter into cornua becomes altogether lost. While some nerves take their origin from or terminate in the central tube of grey matter, in other cases the collections of nerve cells and fibres forming the nuclei of the cranial nerves have become more or less separated from it. Moreover, the central grey matter is by itself quite inadequate to deal with the flood of ingoing impressions entering the central nervous system through the organs of special sense, or to co-ordinate these with one another, or with those arriving from the skin and lower part of the body. In consequence, masses of grey matter, which have no representatives in the cord, make their appearance, and may be regarded as additional sorting or relay stations for the various kinds of impulses which determine the nervous activities of the animal.

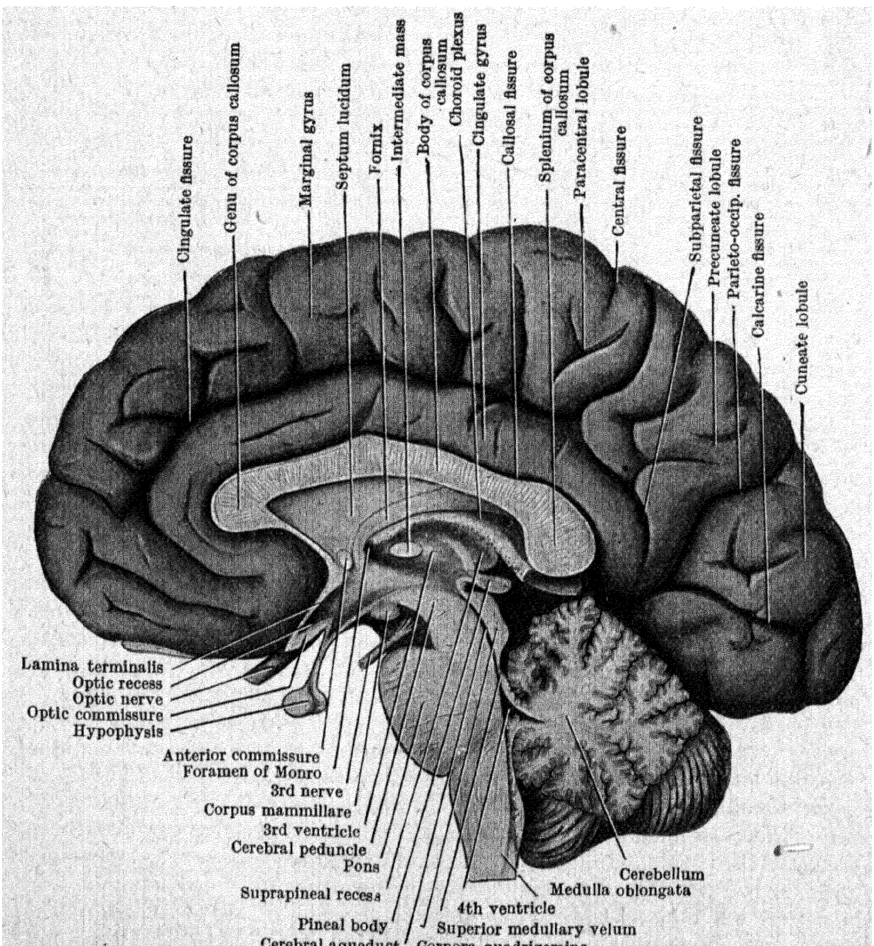

FIG. 130. Medial Section of an Adult Human Brain. (J. SYMINGTON.)

The anatomical relationship between the parts of the central nervous system just mentioned may be gathered from a careful study of Fig. 130. The greater part of the diagram is occupied by the thick, much-convoluted cerebrum, the right and left halves of which are associated by the large mass of axons forming the corpus callosum. Below this will be seen a hollow funnel-shaped mass of tissue which is narrowing as it passes downwards from cerebrum to mid-brain. In this mass the ganglia at the base of the cerebrum are situated. Past them the long path axons travel through the internal capsule, connecting cerebrum with mid-brain. The narrow neck of the mid-brain is well shown. Below this comes the pons which contains the long paths and which by its transverse fibres also associates the two halves of the cerebellum. Below the pons is the medulla, while the laminated cerebellum is seen lying behind.

CENTRES. Often the cells of the grey matter are arranged in groups of cells of similar size and common functions called *neurone pools*, and when such groups are sufficiently conspicuous, either anatomically by their size, or physiologically by their functions, they are usually spoken of as centres. Thus we can distinguish anatomically such groups as the oculo-motor nucleus, the red nucleus, etc., or physiologically a functional group such as the respiratory centre, micturition centre, etc. Those aggregations into physiological groups such as these latter are not necessarily anatomically definable, since they often consist of several closely co-ordinated groups of cells, which may be at considerable distances from one another: the unity is one of function rather than of position. Frequently, however, one group is of predominant importance and of precise location and is more clearly entitled to the name of centre, *e.g.* the cardio-inhibitory centre. Fig. 131 will give an idea of the actual anatomical relationship of different neurone pools.

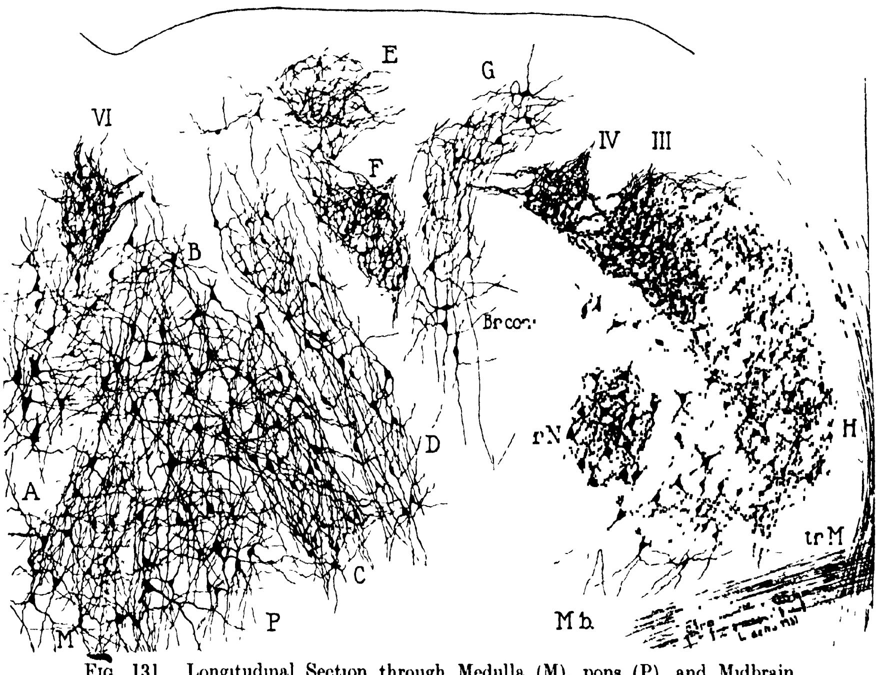

FIG. 131. Longitudinal Section through Medulla (M), pons (P), and Midbrain (M.b.) of Mouse. III, IV and VI, the oculomotor nuclei; *r*.N, the red nucleus, Br.conj., crossing of superior cerebellar peduncles; A, B, C, D and H, various nuclei or neurone pools of the reticular substance, belonging to motor correlation system and having connections with the oculo-motor neurones. E, F and G are neurone pools belonging to the autonomic system; tr.M., tract of Meynert. (LORENTE DE NÓ. *J. Neurophysiol.*, 1938, **1**, 207)

THE AUTONOMIC NERVOUS SYSTEM

In connection with the central nervous system is an important system called the autonomic nervous system. This, which subserves the control of most of the involuntary functions of a vegetative type, *i.e.* those concerned with the routine reflex adjustments of most of the principal systems of the body, is divisible into the sympathetic and parasympathetic systems. The *sympathetic system* is connected with efferent fibres from the thoracic region of the spinal cord, the *parasympathetic* with certain cranial and sacral nerves. The two systems have in common the possession of synaptic connections situated in pools called *ganglia* which lie *outside* the central nervous system.

The work of many of the medullary, and other " centres " which control important bodily functions is largely carried out through the mediation of the autonomic nervous system, which therefore is of much greater physiological significance than its modest anatomical build would lead us to expect.

REFLEX ACTION

The conception of the reflex arc was evolved early in the nineteenth century, though it is chiefly to Sherrington that we owe the initiation of researches which have made it a corner-stone of physiological neurology.

It is common knowledge that the application of many kinds of stimuli to different parts of the body produces an involuntary movement. Thus the hand which touches a hot surface is withdrawn, and this movement is complete almost before we are aware of any sensation. Responses that occur without volition are called reflexes if their execution involves the central nervous system or a part of it. The criterion of a reflex is that it is no longer elicitable if the nervous centres possibly involved are destroyed. Sensation may accompany or follow a reflex action, but many important reflexes occur without the obtrusion of sensation into consciousness, as *e.g.* in the normal blinking of the eyelids at frequent intervals.

These automatic responses are of great variety, involving every system and organ of the body, and in a majority of cases they serve to protect, or otherwise further the interests of, the organism as a whole, or of the species. By their agency the various involuntary actions of the body under innumerable changes of conditions are properly effected and suitably co-ordinated, and its voluntary actions rendered more smooth and efficient. The secretions of glands and the movements of the viscera, the responses of the heart and blood vessels, and indeed all those bodily functions of which we are nearly always unaware, are largely controlled by delicately co-ordinated reflex actions.

Most of our detailed knowledge of reflex action, however, has been gained by the study of muscular responses, for the reason that they are the most objective and easily measured. As an example of a motor reflex in man we may take the knee-jerk. If one sits with, say, the right thigh crossed loosely over the left knee in such a way as slightly to stretch the quadriceps extensor, and then taps the right patellar tendon, a sharp extension of that leg at the knee-joint results. For this, as for all other reflexes, certain component structures are indispensable, and these components when reduced to their simplest terms of a one-fibre track form what is known as a reflex arc.

THE REFLEX ARC

A reflex arc comprises an afferent ' limb,' a centre, and an efferent ' limb ' (Fig. 132).

(1) The *afferent limb* of the arc consists of the receptors, and the afferent neurone. In the case of the knee-jerk the receptors in the tendon and quadriceps muscle are stimulated by the tap. As a result of this they originate nervous impulses which enter the spinal cord by the dorsal roots of, say, the 4th lumbar nerve.

(2) In *the centre* (in our example the 4th lumbar segment of the spinal cord) the impulses pass by a collateral fibre into the dorsal horn of grey

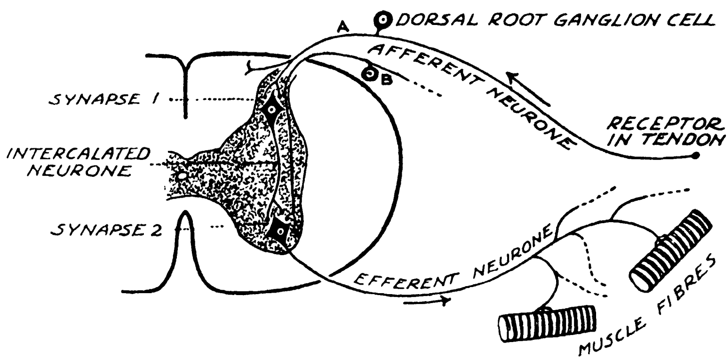

FIG. 132. Diagram illustrating the Reflex Arc in the Knee-jerk.

matter, and so by synapse to a nerve cell, known as an *internuncial* cell (or interneurone or relay cell) which is in turn excited ; the nervous impulse thus relayed passes by a short axon to the ventral horn and there, at a second synapse, excites a large multipolar motor cell. According to some, the afferent fibres concerned in the knee-jerk pass direct to the motorneurones as shown by fibre B.

(3) *The Efferent Limb.* This consists of the motor nerve cell, its axon or motor nerve fibre and the *effector organ* which effects the response. The excited motor cell discharges a fresh relay of impulses along the motor fibres which leave by the ventral root (4th lumbar) and so the excitation is ultimately conveyed to the muscle (quadriceps). The whole process for the knee-jerk occupies about 10 msec., most of which is taken up by the passage of the impulses along the nerve fibres.

We may substitute any other receptor organs, paths, centres and effector organs, but the general principle remains the same as that illustrated above. For example, we may consider the reflex secretion of saliva which follows when an acid is introduced into the mouth, as shown in Fig. 133. Here the afferent nerve is the VIIth cranial, the centre is in the medulla, the efferent path is by the chorda tympani (with another cell station peripherally) and the effector organ is a salivary gland. No doubt internuncial neurones are also placed on the arc.

Since the arc for a particular reflex only involves a limited amount of the central nervous system, it is possible, and often convenient, when studying reflexes in the laboratory, to remove parts of the central nervous system not involved. This is well illustrated in the " spinal frog " in which the brain

has been destroyed, and in which spinal reflexes are easily studied. For example, if the frog is hung up by the head, and a toe of a hind leg is pinched, the leg is promptly drawn up in a co-ordinated flexor movement; if it is held so that it cannot move, the opposite limb moves vigorously instead.

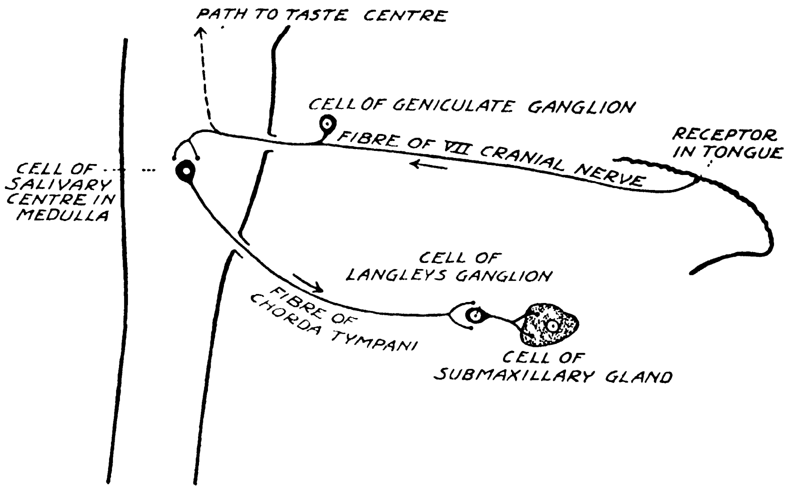

FIG. 133 Illustrating the Reflex Secretion of Saliva

Again, if a scrap of filter paper soaked in dilute acetic acid is placed on one flank, the hind leg of the same side performs accurate movements to remove it; if that leg is held firmly, the opposite leg is brought into action.

The spread, or *irradiation* of a reflex, as shown by this passage to the opposite side, illustrates the fact that alternative paths are available in the central nervous system, and a further spread is seen if both legs are restrained, when the fore legs enter into action.

Integrity of Reflex Arc. The necessity for the integrity of the reflex arc can also be illustrated with the spinal frog. If all the nerves of a limb are divided and the foot of that limb then stimulated, there is no response on either side, because of interruption of the afferent path. If the foot of the opposite side is stimulated that leg will be moved, but if its movement is prevented the denervated limb will not now be affected by irradiation because the efferent path is interrupted by division of its motor nerves. Lastly, if the spinal cord is destroyed with a wire, all reflexes are permanently lost, because the centres are absent. More elaborate experiments which have been performed on higher animals illustrate the same facts in more precise fashion. Thus if in a cat or dog all the dorsal roots or all the ventral roots are divided, no spinal reflexes can be elicited.

Integrity of Reflex Arcs in Man. In the human subject, the existence of certain reflexes supplies evidence for the integrity of the reflex arcs concerned, and is therefore of clinical importance. Thus the presence of knee-jerks shows that the 4th lumbar segment of the cord, with its afferent and efferent nerves, is intact, and in similar ways the following reflexes illustrate the integrity of the spinal segments shown below:—

Segment	Reflex
C 5	Biceps and supinator jerk
C 6	Pronator jerk
C 7	Triceps-jerk
Th 7–12	Abdominal reflex
L 1	Cremasteric reflex
L 4	Knee jerk
L 4–5	Gluteal reflex
S 1	Ankle-jerk
S 2	Plantar reflex
S 4–5	Anal reflex

Other reflexes indicate the integrity of various higher parts of the central nervous system, *e.g.* the contraction of the pupil of the eye to light shows that the IIIrd cranial nerve nucleus and the essential optic and efferent paths are intact.

In disease these reflex arcs may be interfered with in a variety of ways. some of which closely resemble the conditions introduced in laboratory experiments. Thus in tabes dorsalis, knee-jerks and other reflexes are lost because the dorsal spinal nerve roots suffer destruction, throwing the reflex arc out of action by loss of its afferent limb. In anterior poliomyelitis the motor cells of the ventral horn degenerate, which causes paralysis of the affected segments by loss of the efferent limb of the arc. In syringomyelia the grey matter of the cord is generally affected, so that the whole central mechanism of the arc is lost.

CHAPTER XIV

THE RECEPTORS

THE receptors are the structures at which afferent nerve impulses are normally originated in response to appropriate, or adequate, stimuli. They are conveniently subdivided into exteroceptors and interoceptors. The *exteroceptors* convey impressions of the outer world and are again divisible into telereceptors, chemical receptors and cutaneous receptors. The telereceptors are the end organs for vision and hearing, and will be specially considered under special senses as also will the chemical receptors of taste and smell.

The *interoceptors* fall into the three groups of *proprioceptors*, *visceroceptors* and *chemical interoceptors*. The proprioceptors are end organs stimulated by the position or activity of the body itself and visceroceptors similarly are situated in and give impulses arising from the viscera. Chemical interoceptors are present in the carotid sinus and certain other situations.

CUTANEOUS RECEPTORS

(For details, text-books of Histology should be consulted.)

The cutaneous receptors are of two types; viz. the free terminal ramifications and the specialized cutaneous end organs. Some of the principal types are shown in Fig. 134, but there are many others.

FREE RAMIFICATIONS. These are widespread and are usually non-myelinated at their terminations, or even throughout their course. Such terminal ramifications often form unmyelinated plexuses just beneath the epidermis,* and fine branches from this penetrate between or even into the epidermal cells. These free endings almost certainly subserve the pain sense and perhaps in some cases also touch.

SPECIAL CUTANEOUS RECEPTORS. There are many varieties of these, *e.g.*,

RECEPTORS FOR TOUCH. The chief varieties are: the hair follicle, the Meissner's corpuscle, and the Pacinian corpuscle (Fig. 134).

The hair follicles are supplied with a fine plexus of non-medullated terminal filaments, and these are stimulated whenever the hair is moved. *Meissner's corpuscles* are placed in certain of the papillæ of the skin. The fine non-medullated terminal fibres form a spiral imbedded in connective tissue. *Pacinian* corpuscles lie deep in the subcutaneous tissue of the hands and feet, but also in the joints and mesentery. The nerve fibre enters, loses its medullary sheath and becomes surrounded by an oval laminated structure of considerable size.

RECEPTORS FOR TEMPERATURE CHANGE. These are the Krause end-bulbs for cold (Fig. 134) and the Ruffini organs for warmth. In both of these there is a basket-like network of nerve fibres enclosed in an oval connective tissue capsule.

* WOOLLARD. *Brain*, 1936, **58**, 352; *J Anat.*, 1936, **71**, 54.

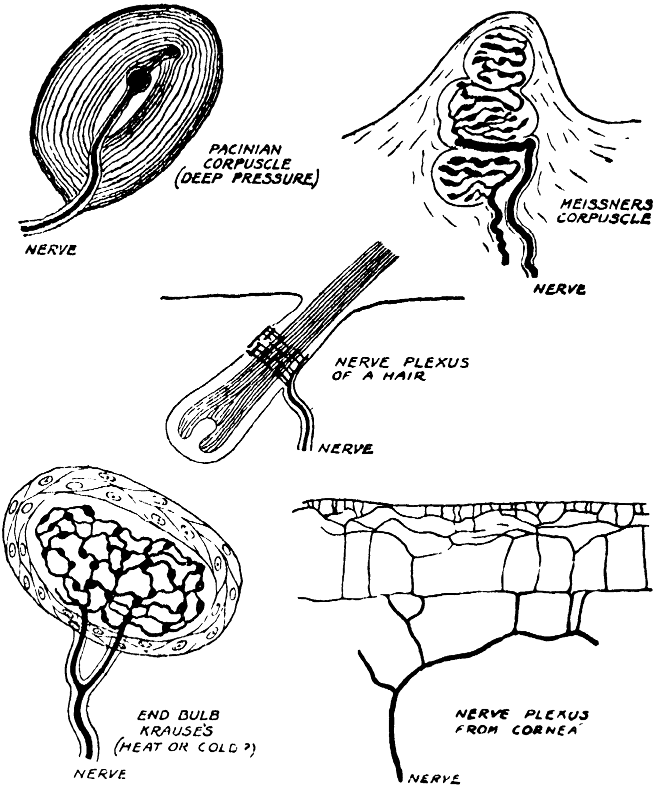

FIG. 134. Skin End Organs It has been supposed that corpuscles of the type of Meissner's corpuscle subserve touch sense, and that nerve plexuses, such as that illustrated for the cornea, are for pain.

THE INTEROCEPTORS

PROPRIOCEPTORS. The impulses from these receptors, often termed *kinæsthetic*, depend upon the movements or position of the body itself. They comprise the labyrinth of the internal ear, and the receptors of muscles, tendons and joints, and upon the impulses they furnish to the central nervous system the accurate pose and movement of the body largely depends.

The labyrinth consists of the *utricle* and *saccule* and the three *semicircular canals*, and will be considered in further detail in a later chapter. The receptors of the utricle and saccule are variously stimulated according to the *position of* the head in space, *i.e.* it is a static register, while the receptors of the three semicircular canals are differentially excited according to the *movement* of the head in the planes of space, and hence its impressions are kinetic in nature. The impulses aroused are conveyed from the labyrinth to the medulla by the vestibular division of the VIIIth cranial nerve.

Muscle Afferents. If the ventral roots supplying a so-called motor nerve are divided and time allowed for degeneration to occur, it is found that as many as one-third of the fibres remain undegenerated, *i.e.* are afferent fibres. The *muscle proprioceptors* are represented by the muscle spindles. In these structures a specialized type of muscle fibre, often split into several

finer fibres, receives three nerve fibres. One is an afferent nerve fibre which, after penetrating the sarcolemma and losing its own neurolemma and medullary sheath, is spirally wrapped round the muscle fibres, the whole structure being enclosed within a capsule (Figs. 135 and 136).

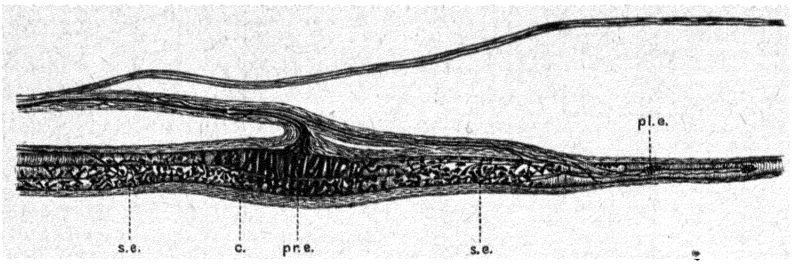

Fig. 135. A Neuro-Muscular Spindle of the Cat. (Ruffini.)
c. Capsule. *s e* Dendritic ending.
pr.e. Spiral ending. *pl.e.* Plate ending.

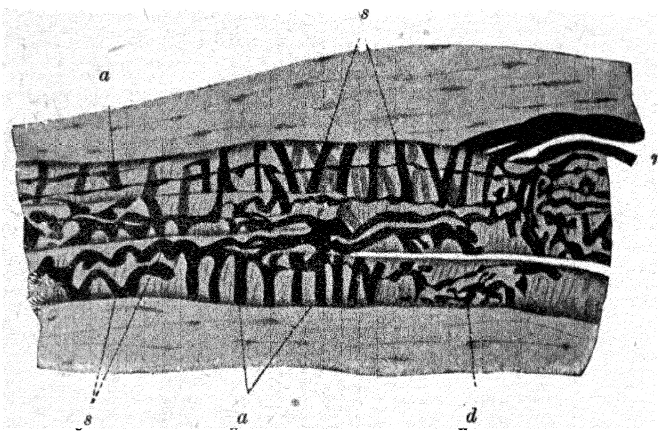

Fig. 136. Part of a Muscle Spindle more highly magnified. (Ruffini
n. Nerve fibres passing to spindle. *s.* Spiral endings.
a. Annular endings of axon. *d.* Dendritic endings.

Usually a second afferent fibre enters the capsule and forms dendritic branchings on the surface of the modified muscle fibres (Fig. 136). That these two types are both afferent is shown by the fact that their specialized endings disappear after removal of the dorsal spinal root-ganglia that supply the muscle in question. The spindle fibres also receive efferent fibres with the usual motor end-plates. They degenerate on section of the ventral nerve roots concerned.

The muscle spindles are regarded as *stretch receptors, i.e.* they are excited when the muscle is elongated passively. When the muscle is contracting, any tension on the spindle fibres would be relieved by the support provided by surrounding fibres in a state of contraction.

The *tendon receptors* are represented by the Golgi tendon nerve-endings, which are found in the tendon near to its attachment to the muscle fibre. They are sensitive to tension, but since they are placed beyond the end of the muscle fibre, they will respond to a pull of any kind on the tendon, whether due to passive stretch or to actual contraction of the muscle. We therefore speak of them as *tension receptors.*

In addition to the above, receptors of other kinds will also be brought into play whenever movement occurs or posture is modified. Thus *Pacinian corpuscles* are found not only in the dermis but in tendon sheaths, joints, &c., and free nerve networks are also abundantly distributed to muscles, joints, tendons, &c.

VISCEROCEPTORS. The viscera and other internal parts of the body are not endowed with sensation in the ordinary acceptance of the term, but can be the seats of origin of a great variety of afferent impulses including in some cases those capable of producing pain. Most of the hollow organs are provided with *stretch receptors* of some kind. These and other visceroceptors are often of the nature of dendritic branchings of non-medullated terminal nerve twigs. A remarkable instance of such a stretch receptor is in the carotid sinuses (Fig. 137), from which afferent impulses arise when the pressure in the carotid artery is increased.

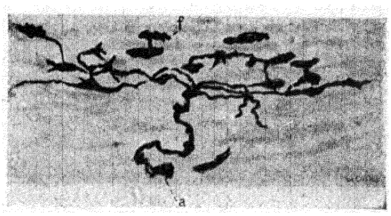

FIG. 137. Sensory endings in the adventitia of the carotid sinus in man: (*a*) arborising nerve fibres; (*f*) end organs. (C. Heymans, *after* de Castro.)

In addition to the stretch receptors, the carotid sinus, or carotid body in particular, is provided with *chemical receptors* which are affected by certain chemical changes in the blood passing through it.

Other important visceroceptors are the Pacinian corpuscles which are widely distributed over the peritoneal cavity.

THE EXCITATION OF RECEPTORS

Since it is the function of the receptors on suitable excitation to originate nervous impulses, we must enquire into the conditions and result of this stimulation.

The conditions and results of stimulation of the receptors differ in several important respects from those of nerve fibres. One difference lies in their specificity, *i.e.* they are so constructed as to respond only, or most easily, to one kind of stimulus, those of the retina to light, those of muscle to stretch, and so on.

Another difference is that the application of a stimulus to a receptor very readily leads to the origination of a *series or train of excitations*, which are duly passed on to the nerve fibre. This property is only feebly developed in nerve fibres themselves, so that stimuli applied directly to them do not, unless very strong, produce more than one nerve impulse per stimulus, even if this be a prolonged one. When a *prolonged steady stimulus* is applied to a receptor, however, a prolonged train of impulses is set up, and passed on to the nerve fibre, since each impulse in the receptor acts as a new stimulus to its nerve fibre.

STRENGTH OF STIMULUS. Since the nerve impulse is an all-or-none phenomenon, the only effect of increasing the *strength of the stimulus* to a given receptor can be to produce a larger number of impulses

along its nerve fibre. This results in the impulses following one another at shorter intervals, and also in the train continuing to be generated for a time after the stimulus has ceased. But another effect enters into the picture under most conditions in which a stimulus is applied to a tissue endowed with receptors, viz. as the stimulus is increased, an increasing number of receptors is excited. Hence an augmenting stimulus normally leads to an increasing rate of discharge along an increasing number of nerve fibres.

On account of this fact, the detection of the impulses initiated by a given stimulus requires, in practice, a special technique, which we owe to Adrian and Zotterman,* and to Adrian and Bronk,† by which the nerve impulses traversing a single nerve fibre are recorded. Adrian and Zotterman achieved this by isolating one receptor (spindle of sterno-cutaneous muscle of the frog) by cutting away the others; Bronk ‡ by dividing all except one of the afferent nerve fibres that led away from the sensory tissue (muscle or skin); Matthews § by using a tiny muscle (of a frog's toe), which had only one spindle.

In each case the spike potentials along the single nerve fibre were recorded, after amplification, and the complication due to the recording of impulses along many nerve fibres was avoided. By similar methods it has been possible to obtain records of the nervous impulses (spike potentials) which pass along many kinds of afferent fibres on appropriate stimulation of their receptors, and in all cases the results are essentially similar (Fig. 138).

FIG. 138. Action potentials (*i.e.* nerve impulses) in a single nerve fibre from a frog's muscle spindle, caused by stretching muscle. The waves are all similar in size, but increase in frequency as the tension increases and then decline slowly when the tension is constant. (TSAI)

When the strength of the stimulus applied to the receptors is raised, the frequency at which they respond is increased up to a certain maximum, which in some receptors may reach as much as 300 per second; this is about the limit at which a nerve fibre can fully respond to direct stimuli, since the absolute and relative refractory periods of the fibre are about 3·5 msec. It follows that the refractory periods of the receptors themselves must at most be of this order.

ADAPTATION. All nervous structures exhibit the phenomenon of adaptation, *i.e.* the responses to a stimulus become less and less frequent with time. Nerve fibres have this property so strongly developed that each application of a stimulus usually only produces a single nerve impulse. With receptors more than one excitation usually results from each stimulus, and they are capable of responding to a continuous stimulation by firing off a long train of responses, which pass on to the nerve fibre. Thus the stretch receptors of muscle, or the touch receptors of the skin, when subjected to appropriate mechanical stimulation fire off a series of nerve impulses into their respective nerve fibres. The receptors, however, do show adaptation, as a result of which the train of impulses, at first in quick succession, soon shows a slowing down in frequency. In this respect the different receptors

* ADRIAN and ZOTTERMAN. *J. Physiol.*, 1926, **61**, 151.
† ADRIAN and BRONK. *J. Physiol.*, 1928, **66**, 81
‡ BRONK. *J. Physiol.*, 1929, **67**, 17.
§ MATTHEWS. *J. Physiol.*, 1931, **71**, 64.

show considerable differences. In muscle receptors the rate at which impulses are generated falls off at first and then reaches a fairly steady rate of discharge (Fig. 139); whereas skin receptors adapt much more rapidly and may in spite of continued stimulation, ultimately only discharge very slowly. This corresponds to our own subjective experience. When the skin is lightly

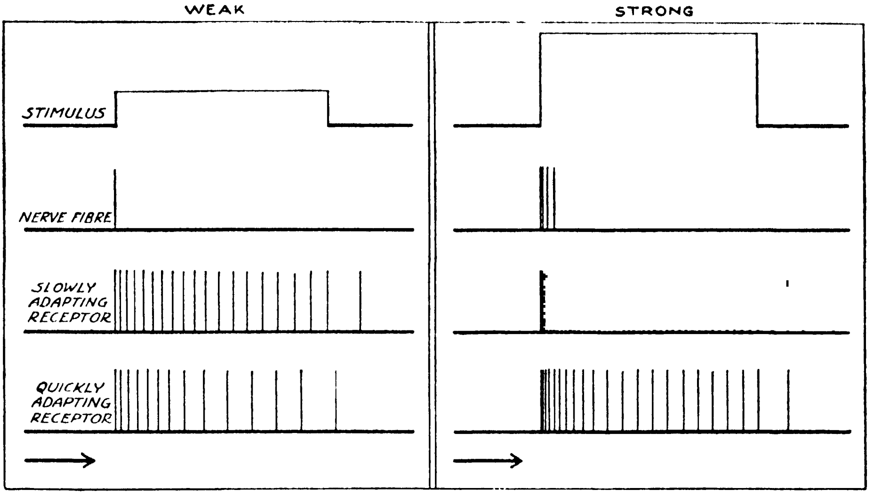

FIG. 139 Diagram illustrating the effects of continuous Stimulation, Weak and Strong, on Nerve Fibre and on Receptors. The vertical lines represent nerve impulses Nerve fibres give only one impulse for weak stimuli or two or three for very strong ones. Receptors discharge trains.

touched, as, *e.g.* by clothing, the sensation is much more intense at first than it subsequently becomes—otherwise the wearing of clothes would be intolerable.

NATURE OF ACTION OF RECEPTORS. Why and how the various receptors respond to their appropriate stimuli, and to those alone, is an interesting question, but one to which no definite answer can at present be given. Whatever the manner in which the state of excitation is brought about, the ultimate result is the same, viz. the generation of trains of nerve impulses along the fibres whose endings they form. It is not improbable, therefore, that the effect on any end-organ of its appropriate stimulus is in all cases the same physico-chemical process. Observations on the retinal terminations suggest that this change is a surface *depolarization*,* and this view would be acceptable, since it brings the process of excitation of nerve-receptors into line with that of other structures, and particularly with that of nerve cells, which like the receptors also generate impulses. Two facts are in accord with this view: artificial depolarization by an electric current causes excitation; and an impulse sent backwards (antidromically) into the receptor by artificial stimulation of its nerve fibre causes the receptor to enter for a short time into a refractory state,† and so interrupts its normal rhythm of discharge.

* HARTLINE. *J. Cell. Comp. Physiol.*, 1932, **1**, 277.
† MATTHEWS. *J. Physiol.*, 1933, **78**, 46.

CHAPTER XV

THE SPINAL CORD

AND ITS RELATIONSHIP TO THE HIGHER PARTS OF THE CENTRAL NERVOUS SYSTEM

It is not strictly possible to consider the central nervous system as a series of separate parts, the functions of which when added together make up the functions of the whole. Rather we should aim at understanding how the entire system functions. Keeping this end always in view, however, much that is of value can be learned by considering the separate functions of the parts. We shall therefore in the first place study the functions of the spinal cord in brief fashion, because it is simpler in structure, and because on that account much can be learned about neural physiology that can subsequently be applied to the understanding of the functions of the higher parts.

THE SPINAL CORD

In man the spinal cord gives off, symmetrically on either side, thirty-one pairs of nerves. Each nerve has two roots, an anterior, or ventral, root and a posterior, or dorsal, root. The ventral roots are efferent (*e g.* motor) in function, and the dorsal ones afferent (sensory). A mixed nerve consists of fibres which are connected to both these roots. The anterior root fibres emerge from the cord in a series of rootlets; the posterior root fibres, on their way into the cord, pass first to the posterior root ganglion and then enter the cord as a compact bundle. On section the cord is seen to consist of an H-shaped core of grey matter surrounded on all sides by white matter The former contains the nerve cells, the latter is made up of medullated nerve fibres which are devoid of neurolemma and are connected into bundles held together by neuroglia.

THE NERVE CELLS OF THE CORD

The nerve cells of the grey matter of the spinal cord are all multipolar cells; they are not uniform in size or distribution, but tend to be aggregated in pools or columns, the cells of each column having approximate similarity of function. The cells are of Golgi types I. and II. The type II., with short axons, are found only in the dorsal horn, and chiefly in its lateral basal group. Their short axons pass towards the ventral horn of the same or the opposite side. The type I. cells usually send out their axons into the tracts of the white matter. Important groups of the posterior horn are the cells of the *substantia gelatinosa Rolandi*, and at the base of the posterior horn is the *Clarke-Stilling column* (or mesial basal group). The cells of the substantia gelatinosa Rolandi send out axons mainly into the spinothalamic tracts of the opposite side. The axons of the cells of the Clarke-Stilling column pass out into the dorsal and ventral spino-cerebellar tracts and so reach the cerebellum, mainly of the same side. (Fig. 143.)

In the lateral portions of grey matter, in the thoracic and sacral regions, are cells forming the *lateral splanchnic column* which give origin to axons that pass out *viâ* the ventral roots and white rami communicantes, to the sympathetic chain. (Fig. 143).

In the ventral horn of grey matter are three or four groups of cells forming the *motor cell columns*. Their axons pass out into the ventral spinal nerve roots.

THE WHITE MATTER

The white matter of the spinal cord consists of medullated fibres, devoid of neurolemma, and held together by neuroglia. It represents the conducting matter of the cord and contains fibres of four types : (i.) long path afferent axons, such as those of Goll and Burdach in the posterior columns, forming the main tracts to higher parts, (ii.) long path efferent axons, leading down from higher parts, *e.g.* the pyramidal tracts, (iii) association or intrinsic axons, which connect neighbouring segments of the cord, (iv.) commisural fibres which connect opposite halves of the same segments of the cord.

Much of the white matter is divisible into *tracts*, consisting of long fibres of similar function and destination, and these again are distinguished as *afferent and efferent tracts*. The tracts are mapped out mainly by the degeneration method. If a tract is divided, the parts of the axons cut off from their cells will degenerate, *i.e.*, right up to the next synapse, and after some days may be distinguished by appropriate staining methods. The same thing happens if the whole spinal cord, or other part of the central nervous system, is cut across; the axons from cells below the lesion (ascending tracts) will be degenerated in the part above the section, and those with cells of origin above the lesion (descending tracts) will be degenerated only below the section (Fig. 140). If the dorsal nerve root be divided proximal to the ganglion, *i.e.* between the ganglion and the cord, it will be clear that the whole of the course of the axon within the cord will degenerate, and this method provides a way of differentiating those efferent tracts which are directly derived from the dorsal root fibres (A in Fig. 140) from those of secondary origin in the grey matter of the cord (B in Fig. 140).

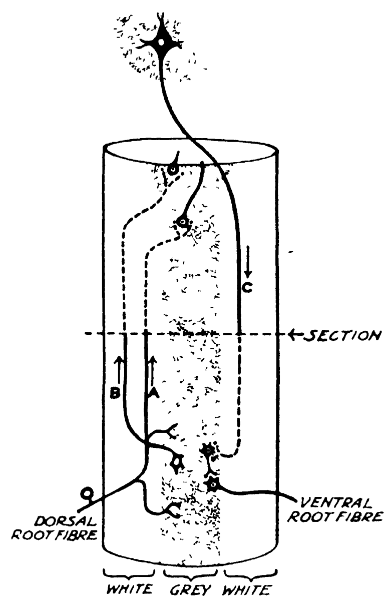

FIG. 140 Diagram to illustrate Method of tracing Tracts by Degeneration A, fibre of an exogenous ascending tract; B, fibre of an endogenous ascending tract, C, a descending fibre. Degenerated tracts shown by broken lines

The cells of origin of divided tracts undergo chromatolysis. Hence the pool of cells from which a tract arises (*e.g.*, tract B in Fig. 140) can often be identified.

The chief paths of physiological importance are briefly as follows :—

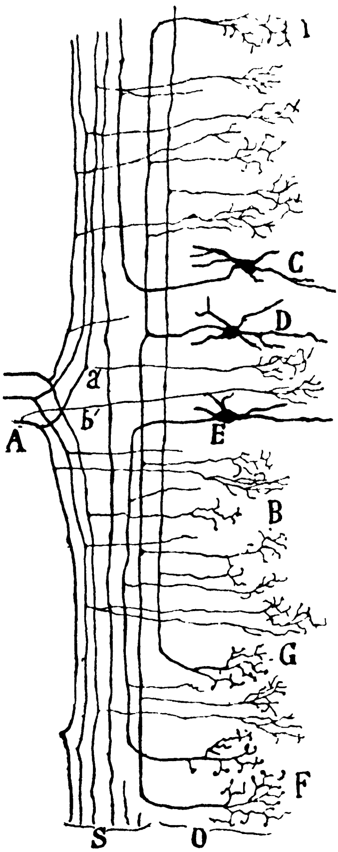

FIG. 141. Longitudinal Section of Spinal Cord of Chick, showing bifurcation of Dorsal Root Fibres and the passage of their Collaterals into the Grey Matter. Three cells of the Dorsal Horn are also seen sending their axons into the dorsal columns of white matter .(short intraspinal neurones). (CAJAL.)

I. **AFFERENT PATHS.** Each dorsal root fibre, on entering the cord, gives off a short descending branch and an ascending branch (Fig. 141), each of which has numerous small collaterals which enter the grey matter of the dorsal horn, and there form synaptic connections with cells in one of the pools. The ascending branch may, (*a*) after a short course, similarly terminate in the grey matter, by synapse with a cell from which an axon arises, to pass out again into the white matter as a fibre of a secondary tract; or (*b*) pass directly as an 'exogenous' tract into the white matter of the posterior columns (Fig. 143). On entering the spinal cord the fibres become grouped into tracts according to their destination and functions.

It has been argued by Stopford * that the afferent nerve fibres fall naturally into two groups. One group is connected with defensive functions, is phylogenetically the older, gives sensory responses of an all-or-nothing character as regards their affective qualities, and has important central connections with the thalamus This group of fibres crosses in its path in the cord. It includes sensations of pain, of extremes of temperature, and of pressure. The other group, less old, uncrossed in the cord, is essentially cortical in its terminations, and is concerned with finer discriminations of localization, discrimination of position and passive movements, fine differences of temperature, and appreciation of lightest forms of touch.

Fig. 142 shows the main tracts of the spinal cord and Fig. 143 illustrates the mode of formation of the chief afferent paths, which is as follows :—

(1) AFFERENT IMPULSES RELATED TO MOVEMENTS. These may be conscious, *i.e.* give rise to sensations, or unconscious; most of the latter are related to the maintenance of posture.

(*a*) The impulses subserving *conscious sensations of movement* enter at the posterior root, ascend in the columns of *Goll and Burdach* until they reach the *nuclei gracilis* and *cuneatus* in the medulla. [The cells in these nuclei give off second order relay fibres which, crossing in the sensory decussation, ascend in the mesial lemniscus (fillet) to the optic thalamus. Third order fibres originating in this convey the impulses to the cerebral cortex.]

(*b*) The *unconscious kinæsthetic impulses*; (i.) those from the skin enter the posterior root, and ascend in the columns of *Goll and Burdach*

* STOPFORD. 1930. "Sensation and the Sensory Pathway." (London, Longmans.)

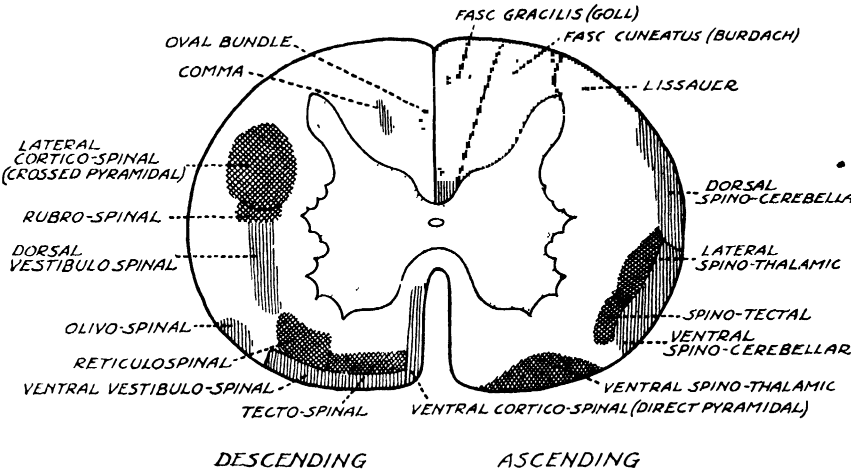

FIG 142. Diagram of the chief Long Tracts of the Spinal Cord. Descending tracts on left, ascending ones on right. (Single hatching, homolateral, cross-hatching heterolateral)

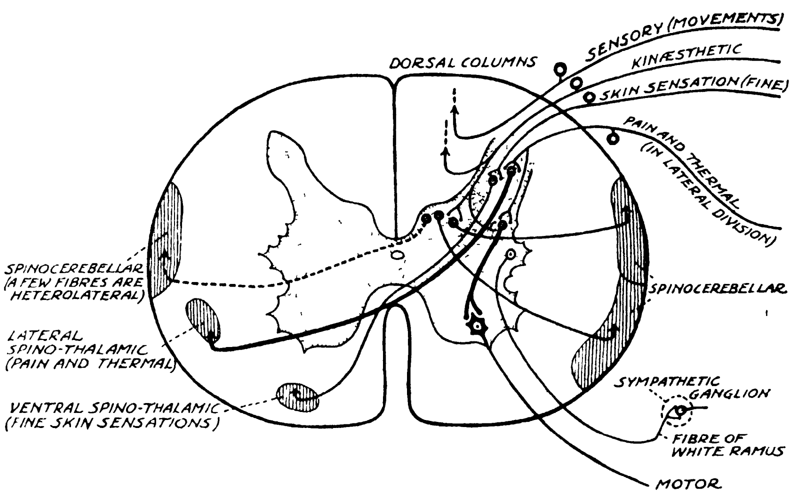

FIG. 143. Diagram illustrating the origin of the chief Afferent Tracts of the Spinal Cord.

to the *nuclei gracilis* and *cuneatus*. [Second order fibres originate from the cells in these nuclei to travel as superficial or deep arcuate fibres to the inferior cerebellar peduncle by which they reach the vermis. Third order fibres may then convey the impulses to the cerebellar cortex.]

(ii.) The *spino-cerebellar tracts* also convey kinæsthetic impulses that do not enter consciousness. They are from muscles, tendons, joints, &c., and most of them reach the cerebellum of the same side. They enter the posterior root and go to Clarke's column; from the cells in this column second order fibres ascend in both the spino-cerebellar tracts, chiefly of the same side. [Those of the *dorsal* tract entering by the inferior cerebellar peduncle, go to the vermis, from which third order fibres convey the

impulses to the cerebellar cortex; a few of the axons from the Clarke's column cells cross to the spino-cerebellar tracts of the opposite side. The *ventral* spino-cerebellar tract, at the level of the mid-brain, turns back abruptly to enter the superior cerebellar peduncle, where the fibres reach the vermis on the same side as that on which they started. From this, third order fibres travel to the cerebellar cortex.]

(2) PAIN AND THERMAL SENSE. The sensory fibres for pain and temperature enter by the *lateral* divisions of the dorsal nerve roots. Many of the fibres are non-myelinated.* They ascend a short distance in Lissauer's tract and relay in the cells of the *substantia gelatinosa Rolandi*. Second order fibres originating in these cells cross near the central canal to form the *lateral spino-thalamic* tract. [In this they ascend by the spinal lemniscus to the optic thalamus, where those concerned with the cruder sensations end. Some others for less crude forms relay round third order cells in the thalamus, which convey the impulses to the cortex.]

(3) FINE SKIN SENSATIONS, *e.g.* light touch, light pressure, fine localization, pass up at first in the *dorsal columns*, and some continue there to reach the nuclei gracilis and cuneatus. Most of them, however, sooner or later end synaptically at cells of the posterior horn, from which axons pass out to the *ventral spino-thalamic* tract of the opposite side. [This joins the spinal lemniscus and ends in the thalamus.]

(4) SHORT PATH DESCENDING fibres derived from the descending axon branches include: a triangular bundle found in the lower lumbar region; the oval bundle of Flechsig, found in the lumbar region; and the comma tract, found in the thoracic region.

(5) AUTONOMIC afferent paths.

THE NOCIFENSOR SYSTEM OF NERVES

It is well known that the skin around an injured spot, *e.g.* an insect bite, or a small burn, becomes tender, and also that an area of tenderness is found in the cutaneous distribution of the maxillary nerve, following stimulation or inflammation of the mucous membrane of the maxillary antrum, or of the teeth in the upper jaw, etc.

Experiments by Lewis led him to conclude that the skin is supplied with a special freely branching system of nerves, which he calls *nocifensor* nerves, because they are associated with local defence of the tissues against injury, and that it is owing to these nerves that the areas of tenderness are produced. These nerves, though connected with the posterior roots, are not sensory in function. When stimulated, they cause changes in the surrounding area of skin, as a result of which the skin becomes tender and hyperalgesic, its pain fibres more readily responding to slight stimuli. The change brought about in the skin is believed to be due to the release from its cells of a substance which acts on the overlapping endings of neighbouring pain fibres, so producing an area of tenderness around the lesion.

If a small area of skin be injured, as by crushing with the point of a forceps, an area of hyperalgesia (tenderness) appears round the damaged spot, spreads slowly for the next ten or twenty minutes over several centimetres around, and lasts up to several hours. If a small cutaneous nerve supplying an area of skin, or if the small area itself, be anæsthetised by a local injection of novocain, and the injury then made within the anæsthetised area, no tenderness or pain appears in or around the insensitive part so long as the damaged part is anæsthetic, but so soon as the anæsthetic wears off, then the tenderness appears in and around the area of damage.† Once the hyperalgesia has spread, it cannot be abolished by subsequent local anæsthesia of the damaged spot alone. Similarly, if the injury is made just outside an anæsthetised area of skin,

* RANSON. *Arch. Neurol. Psych.*, 1931, **26**, 1122.
† LEWIS. *Clinical Science*, 1936, **2**, 373, *B.M.J*, 1937, *i*, **4**, 431, 491.

hyperalgesia spreads at once into the unanæsthetised regions, and only invades the insensitive area as the anæsthesia recedes from it. Electrical stimulation of the *distal* end of a cutaneous nerve (after blocking it above with novocain) also causes development of the tender area in the zone of distribution of the nerve, as soon as the anæsthetic wears off; but stimulation of a nerve *central* to a blocked stretch, though it causes pain, is not followed by hyperalgesia. Removal of the sympathetic supply to the skin does not alter the response.

There is reason to believe that the hyperalgesia is related to the vascular 'flare' which also appears over a similar area around a site of damage to the skin (Chapter XXXIV). The phenomenon, like that of the flare, is probably an axon reflex, as the above facts suggest, but ordinary sensory fibres cannot be involved in it, because of its spreading nature, whereas sensation, including that of pain, is more precisely localised in the skin.

The substance which is supposed to be liberated is not identical with that concerned in causing the flare; the latter is due to the liberation of a histamine-like substance but the nature of the pain-producing substance is unknown.

II. **EFFERENT PATHS.** IMPULSES FOR VOLUNTARY MOVEMENT. (1) *The cortico-spinal tracts* start in the Betz cells of the precentral motor cortex, pass down through internal capsule, crura and pons to reach the medulla. Here the majority of the fibres cross, forming the *motor decussation*; part descend uncrossed. The crossed fibres form the *lateral cortico-spinal* or crossed pyramidal tract; they end at ventral horn cells either directly, or by first synapsing with a cell in the dorsal horn; the axons of the ventral horn cells convey the impulses to the muscles. Those which do not cross travel in the *anterior cortico-spinal* or direct pyramidal tract, but cross near their terminations to reach anterior horn cells of the opposite side from which fibres convey the impulses to the muscles. Thus, all fibres from the right motor cortex are distributed to the left side of the body. The axon leading from the cortex to the spinal motoneurones is called the "*upper motor neurone.*"

In the lower mammals, impulses for posture and group movements probably travel largely in

(ii.) The *rubro-spinal* tract. This arises in the large-celled portion of the red nucleus. The fibres cross to the opposite side, and descend till they reach different levels of the cord, where they finish at anterior horn cells. These give off motor fibres to the muscles.

By means of the rubro-spinal tract the final common motor path is put into communication with the cerebellum of the *same* side (because this cerebellar path crosses twice, once in the superior cerebellar peduncles, and once in the rubro-spinal tract decussation). It also establishes connection between the corpus striatum and the final common paths of the opposite side. The cerebellum and corpus striatum also exert an influence on the final common path through

(iii) The *reticulo-spinal tract.* This arises from the reticular formation of the pons and ends at the anterior horn cells.

(2) EQUILIBRIUM AND POSTURE CONTROL. Impulses travel from the hair cells of the vestibular organs, and from other sources, to *Deiter's nucleus.* The cells in this nucleus give off fibres which descend by the *vestibulo-spinal* tract to the anterior horn cells in the cord. From these, fibres convey the impulses to the muscles. The rubro-spinal and reticulo-spinal tracts are also important efferent paths for postural reflexes.

(3) VISUAL REFLEX MOVEMENTS. As the result of light falling on the retina, impulses are transmitted to the *superior corpora quadrigemina* (*colliculus superior*). Thence, fibres proceed downwards and cross at Meynert's tegmental decussation, forming the *tecto-spinal tract* by which they reach the anterior horn cells, and so reach the muscles. The face, arm and neck reflexes served by this path serve to protect the eyes.

(4) Sympathetic efferents, *e.g.* (*a*) the pupil-dilating fibres. These fibres probably originate in or near to the superior corpora quadrigemina. They descend as far as the eighth cervical or first dorsal nerve roots. Here they probably relay at lateral horn cells, from which white rami proceed out at the anterior horn to travel to the first thoracic ganglion. They then travel up the sympathetic chain to branch off to the eyeballs, which they enter as either short or long ciliary nerves, and then travel to the dilator muscle of the pupil.

(*b*) The vaso-motor fibres. These travel down the cord from the vaso-motor centre in the medulla by an unknown route. They relay in the lateral horn cells. Fibres from these cells pass out at the ventral roots of Th 1 to L 2, forming part of the white rami communicantes to the sympathetic.

There are many other sympathetic efferents (*v.* Chapter XXIII).

EFFECTS OF SECTION OF CORD AND ROOTS

COMPLETE SECTION OF THE CORD. Complete section of the cord results in permanent loss of voluntary movement of all muscles below the lesion. Thus, a monkey which has suffered a section of its lower cervical region will have paralysis of the lower limbs and part of the trunk, while parts above the section are apparently quite normal. The animal may contentedly direct its gaze through the window or may amuse itself by catching flies on the pane. There is also complete loss of all true sensations in parts of the body below the lesion; reflexes such as micturition and defæcation, become automatic, after a period of paralysis; and there is temporary vaso-dilatation below the lesion. These effects will be described later.

HEMI-SECTION OF THE CORD. The symptoms that follow division of the right or left side of the cord were first described by Brown-Séquard, and, after a preliminary period of spinal shock, are: (1) complete paralysis of muscles below the lesion on the same side; (2) increased postural tone and reflexes in muscles below the lesion on the same side; (3) pain, temperature, and some sensibility to touch is lost on the *opposite* side below the lesion; (4) kinæsthetic sensations are lost on the same side below the lesion; (5) temporary vaso-motor paralysis, and therefore a higher temperature below the lesion on the same side. In addition, as a result of irritation to the cut sensory fibres, there may be pain referred to parts of the body below the section. It is referred to the *opposite* side of the body to that on which the lesion of the cord has occurred. If the lesion has destroyed a length of the spinal cord, then in addition to the above we shall have the symptoms of damage to the ventral and dorsal nerve roots.

SECTION OF THE DORSAL ROOTS causes (most clearly when several consecutive roots are cut): (1) loss of all sensations from the limbs or other parts supplied; (2) weakness and inco-ordinated movements of the muscles owing to inability to receive kinæsthetic sensations from them; (3) injuries to the skin of the limbs supplied, owing to the absence of protective sensory impulses; (4) complete loss of tone in muscles of limbs supplied, since the kinæsthetic impulses which are responsible for them do not reach the anterior horn cells. If the section has been between the posterior root ganglion and the spinal cord, the degenerative changes affect the fibres entering the cord. These results are found in the disease tabes dorsalis. If, on the other hand, the lesion has affected the fibres between the posterior nerve roots and the sensory end organs, the fibres degenerate between the cut and the sensory end organs (see Fig. 144).

The above statement regarding the direction of degeneration of fibres after section of the dorsal roots is only an approximate one. Actually many fibres remain undegenerated in the central stump, after section proximal to the ganglion,* and it has also been shown that efferent impulses, detectable electrically, pass outwards along the

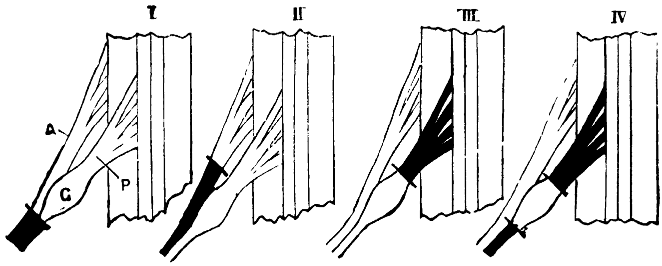

FIG. 144. To illustrate the extent and direction of degeneration as a result of Section of the Spinal Roots. (From YEO.)
Division of: I, Whole nerve trunk, below ganglion; II, anterior root; III, posterior root above ganglion; IV, posterior root above and below ganglion.

dorsal roots.† One among several explanations is that given by Barron and Matthews,‡ viz. that fibres enter the cord by one rootlet, and, after passing a short distance up the dorsal columns, a branch emerges by another rootlet, and passes to a second receptor at the periphery: there is the usual ganglion cell on the course of the normal afferent fibre, but none on the recurrent collateral.

SECTION OF THE VENTRAL ROOTS (several consecutive ones) causes in the part supplied: (1) complete loss of tone in muscles; (2) complete paralysis of muscles; (3) temporary vaso-dilatation owing to cutting fibres which emerge as the white rami communicates to the sympathetic system; (3) nutritional disturbances, *e.g.* disuse atrophy of muscles, &c., unless measures be taken to prevent this. The degenerative changes will affect the nerve fibres between the cut and the muscles.

FUNCTIONAL LEVELS IN THE CENTRAL NERVOUS SYSTEM

Much of the investigation of the functions of the central nervous system involves the operative cross-section of the nervous axis at particular levels, or the removal of portions of the central nervous system. Thus a section may be made at any required level of the spinal cord, medulla or mid-brain; or part or the whole of the cerebral hemispheres, the cerebral cortex, the cerebellum, &c. may be extirpated. Animals so treated represent fundamental preparations for the study in simplified form of central nervous functions.

When the spinal cord is cut in the cervical region we have the *spinal preparation* §; when the section is made at the mid-brain we have the so-called *decerebrate preparation*; if only cerebral cortex is removed, a *decorticate preparation*, and so on.

When a lower part of the central nervous system is separated by section from a higher part, two consequences ensue. First there is *loss of certain functions* of a higher order by reason of interruption of the paths by which the lower centres are controlled from the higher ones. So, for instance, on

* HINSEY. *J. Comp. Neurol.*, 1934, **59**, 117.
† BARRON and MATTHEWS. *J. Physiol*, 1935, **85**, 73
‡ BARRON and MATTHEWS. *J. Physiol*, 1935, **85**, 104.
§ The term 'preparation' in physiology means a surviving portion of an animal.

section at the mid-brain, there is loss of voluntary movement in the trunk and limbs. Secondly, certain new properties may appear as a result of what is known as '*release of function*.' For instance, after mid-brain section there is an exaggeration of the tonus of muscles, particularly extensor muscles, normally employed in the maintenance of posture. This result appears to be due to the removal of a restraint, normally exercised by the higher centres on centres in the medulla, thus revealing or releasing a reflex exaggeration of tone in the extensor muscles (decerebrate rigidity). When the higher centres are removed, the lower ones are released from their control, as first suggested by Hughlings Jackson in 1884. It is probable that primitive behaviour responses of the lower and phylogenetically older parts of the central nervous system are largely dominated and repressed by the higher levels of the nervous system.

THE SPINAL ANIMAL. In the spinal animal proper, section is made at the junction between the spinal cord and the medulla, and often the entire head is removed, the body and limbs then being known as a '*decapitate*' *or* '*spinal*' *preparation* (Fig. 190, p. 290). Since breathing ceases, artificial respiration by means of a pump is carried out, and the preparation then remains alive for many hours, and provides a valuable object for acute laboratory experiments.

When section is made at lower levels, we speak of an animal with *spinal transection* ; if section is above the fifth cervical segment, however, artificial respiration will have to be maintained if the animal is to survive for more than a few minutes, and this is inconvenient. It is customary, therefore, where possible for spinal transections to be made at lower levels, so that the cervical cord, which gives origin to the phrenic nerves, will remain in connection with the higher parts of the nervous system, from which respiration is controlled. Such animals, or human subjects with lesions, *e g.* by bullet wounds, &c., in the dorsal or lumbar cord, may survive for months or years, and provide objects in which the reactions of the spinal cord may be studied without the interfering influences from higher parts.

For a variable length of time after the transection of the cord a condition of depression prevails at levels supplied by the cord below the section. This state of depression is called *spinal shock.* In lower vertebrates such as the frog, it may pass off in a few minutes, but in higher animals it may last for hours, days, or, in man, weeks.

Cause of Spinal Shock. Spinal shock was formerly thought to be a result of the irritation due to the section, but is now recognized as a release phenomenon, due to the cessation of a stream of impulses from above, which normally have a facilitating action.* The reasons for this belief are (1) that if, after the shock has passed off a second section is made lower down, no shock results, (2) that if a ring of the upper spinal cord be cooled, so that conductivity is abolished at that level, shock develops although no irritation is caused by the cooling, and when the cooling agency is removed the shock vanishes again,† (3) that, if the cord is only partly divided, evidence of shock only appears when the vestibulo-spinal and reticulo-spinal tracts (in cats) ‡ or the cortico-spinal tracts (in man) are sectioned.

Alteration in the reflex excitability of the spinal cord is not entirely confined to the parts of the cord below the section, since the parts above the section often show an increased excitability of reflexes (the Schiff-Sherrington phenomenon). This may be

* LIDDELL. *Brain*, 1934, **57**, 386.
† TRENDELENBURG. *Pflüger's Arch.*, 1910, **135**, 469.
‡ FULTON, LIDDELL and RIOCH. *Brain*, 1930, **53**, 311, 327.

attributed to release from an inhibitory influence of the lower segments of the cord on the upper segments.

Spinal Reflexes. During spinal shock there is paralysis of many reflexes, the limbs are flaccid, the blood pressure low, and the bladder fails to empty. All extensor reflexes are depressed.* The flexor reflex and the knee-jerk, however, are usually retained. The *flexor reflex* is obtained when a *nociceptive stimulus* (*i.e.* a stimulus which in a conscious animal would cause pain) is applied to a hind foot or leg, and consists of a flexion of the same (or ipsilateral) limb at all joints (Fig. 145). The *knee-jerk* is obtained by tapping

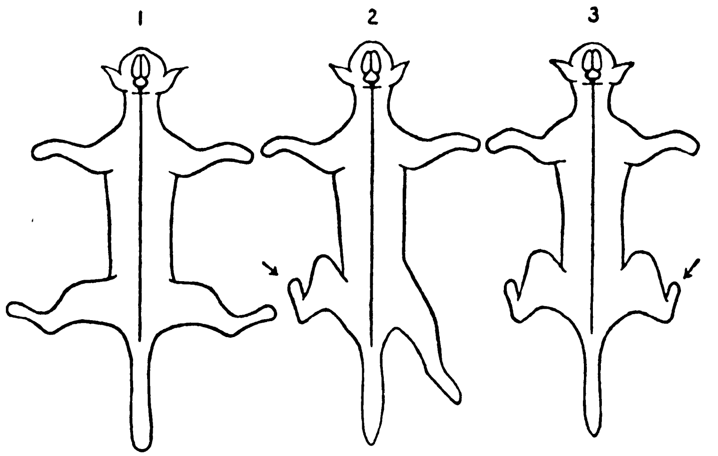

FIG. 145 Diagram illustrating Reflexes in the Spinal Cat (1) The initial pose of the spinal animal. (2) Stimulation of left hind foot causes ipsilateral flexion and contralateral extension in the hind limbs (3) On simultaneous stimulation of both feet there is flexion of both legs, since flexion reflex is prepotent (SHERRINGTON. "Integrative Action of the Nervous System" Constable, 1906)

the patellar tendon while the quadriceps extensors are put slightly on the stretch by the weight of the leg, and consists of a strong extension of the leg at the knee joint.

As the condition of shock wears off, other reflexes make their appearance. Among these are the extensor reflexes The *crossed extensor reflex*, *i.e.* extension in the opposite (contralateral) hind limb is obtained, together with the ipsilateral flexion reflex, when a nociceptive stimulus is applied to the foot or to any afferent nerve from the leg (Fig. 145 (2)). This is clearly a co-ordinated response, enabling the animal to throw more weight on one leg when the other is injured and therefore flexed out of the way. Various *stretch reflexes* also return, but these will be discussed later.

The *extensor thrust reflex* in spinal animals is only seen after recovery has made considerable progress. It consists in a sharp extensor push of the leg when firm pressure is applied to the pad of the foot. Flexion of the opposite leg accompanies it and the two actions represent a compound reflex which forms the basis of galloping.

Another still more complex reflex late in returning is the *scratch reflex*, well seen in the spinal dog. If an appropriate stimulus is applied to a certain

* In acute experiments the extensor reflexes emerge earlier if an intravenous injection of ephedrine (1 mg./kg. for dogs) is given. HINSEY, RANSON and ZEISS. *J. Comp. Neurol*, 1931, **53**, 401.

area (Fig. 146) of skin over the flank and shoulders, the ipsilateral hind limb executes rapid scratching-movements, while the contralateral limb is rigidly extended. The appropriate stimulus should be a continuous one, slowly moved along among the hairs, and the significance of the reflex is self-evident.

Isolated Segments. For some investigations it has been found possible to isolate short portions of cord consisting of one or two segments only, by

THE SIMPLE REFLEX

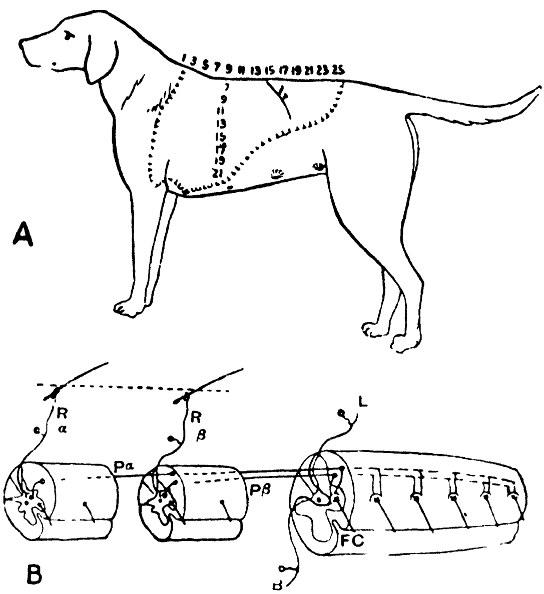

FIG 146 A, the area from which the scratch-reflex can be evoked B, the reflex arcs involved. (SHERRINGTON. "Integrative Action of the Nervous System." Constable, 1906)

making sections above and below. In portions consisting of only two or three segments, quite normal reflex responses can be obtained.*

THE DECEREBRATE PREPARATION. Division of the nervous axis at the level of the mid-brain (and destruction of the parts above the section) give a very valuable preparation. In this, the body is left under the management of the spinal cord, medulla, cerebellum and pons, with variable parts of the mid-brain, according to the level of the section. It is sometimes called a 'bulbo-spinal' preparation. As with the decapitate preparation, after the operation is completed no further anæsthetic is required, because the higher centres are all removed; unlike the decapitate preparation, various complex automatic and reflex acts are preserved. Thus respiration continues, swallowing and vomiting can take place, the arterial blood pressure remains high, and various cardiac vascular and respiratory reflexes are retained. Temperature regulation and righting reflexes are lost, however, as in the spinal animal, but, on the other hand, there is nothing corresponding to spinal shock. It is clear, therefore, that the nervous mechanism of temperature regulation is situated higher than the level of

* TEN CATE. *Arch. néerl. Physiol*, 1932, **17**, 331, 525.

the mid-brain, but that the cells of origin of those tracts, division of which causes spinal shock, must be somewhere between the upper spinal cord and the mid-brain. Not only does the decerebrate animal *not* show the transitory extinction of extensor reflexes characteristic of spinal shock, but instead, it shows such great exaggeration of all extensor reflexes that many of the extensor muscles are aroused to a state of permanent hypertonus. The flexor responses seen in the spinal preparation can all be elicited, however, though not so easily.

The extensor tonus is evident as a rigidity of the limbs,* spoken of as *decerebrate rigidity* (Figs. 147 and 190, p. 290). It affects the anti-gravity muscles, *i.e.* those muscles involved in keeping the animal in a normal standing posture, which in most quadrupeds are the extensors at all joints

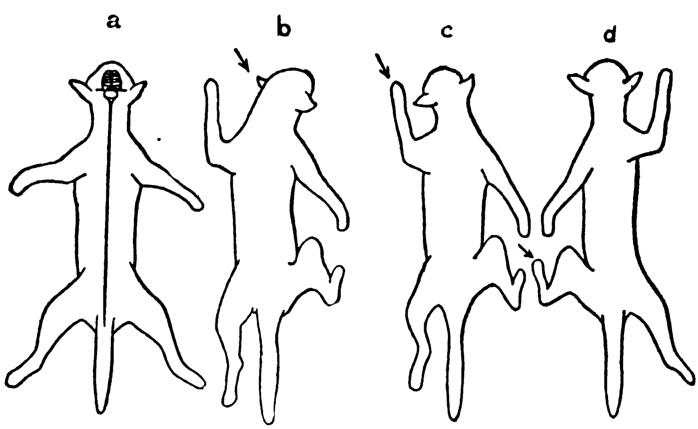

Fig. 147. Reflex Figures in Decerebrate Cat (a) Normal decerebrate rigidity—extension of all limbs. (b) Stimulation of left pinna—flexion of left fore and right hind limbs, increased extension of others. (c), (d) Stimulation of left fore and left hind limbs respectively. (SHERRINGTON.)

of the four limbs, and the muscles that keep the head and tail from falling forwards.

If we accept spinal shock as a release phenomenon, due to the removal of an influence from above which augments the extensor reflex responses, then we must recognize decerebrate rigidity as due to the retention of that influence. It is thus a release phenomenon of another type. We may suppose that the influence of centres in the medulla, or other higher parts that are present in the preparation, in exaggerating extensor responses is now left in undisputed possession of the field, whereas in the intact central nervous system the effects of these centres are subordinated to still higher centres. Hence a grossly exaggerated picture of reflex standing results, which under the influence of higher centres would be modified to give the normal stance.

The decerebrate preparation is accordingly very useful for the investigation of phenomena of extensor reflexes and their inhibition. It also shows many instances of complex reflexes involving co-operation between different segments of the cord. For instance, there is the phenomenon of *reflex figures, i.e.* if a pinna or one limb is stimulated characteristic flexion and extensions of limbs result as shown in Fig. 147 (b).

* SHERRINGTON. *J. Physiol.*, 1897, 22, 319.

It will be clear that these various reflex figures play their parts in the normal movements of the animal.

SPINAL TRANSECTION IN MAN

The consequences of spinal transection in man have been studied in great detail by Head and Riddoch * on men wounded in the Great War. At the moment of injury, if the transection is complete, the subject feels that the lower part of his body has vanished. The patient will never recover, though his condition alters as times goes on. (I.) There is first a prolonged *stage of shock*, with complete loss of movement and sensation in the parts below the lesion, and a feeling of a tight or painful girdle round the body is often present just above it. The muscles are flaccid, the limbs cold, and there is no sweating below the lesion. Blood pressure is low. Nearly all the muscular reflexes vanish. The anal and bladder sphincters are tightly contracted, and urine and fæces tend to be retained. This stage lasts for two or three weeks

(II.) In the second stage there is a *return of reflex activity*. The bladder sphincters become less tonic, though the bladder is not reflexly emptied. The arterial pressure returns to normal and there is some improvement in circulation in the legs. Tone begins to return a little in the flexor muscles and flexor reflexes appear, the first hint of this usually being the occurrence of slight upward movement (' dorsiflexion ') of the big toe (the Babinski reflex),† when the sole of the foot is scratched Later, the complete flexor reflex (dorsiflexion of toes and of foot, flexion of knee and hip with abduction of thigh), slowly emerges.

The Babinski reflex is a primitive one which is absent from normal subjects : in these the response of the toes is plantar-flexion. This is converted to the dorsiflexor response in any circumstances in which the cortico-spinal tract is severed It is unfortunate that the name ' extensor response ' has been attached to the Babinski reflex. Dorsiflexion of the big toe, with spreading of the other toes as seen in the Babinski response, are part of a generalized flexor reflex ‡ The extensor longus hallucis, since it shortens the limb, is physiologically a flexor muscle.

The abdominal reflex, a contraction of the recti on scratching the surface of the abdomen, is another conspicuous flexor reflex.

Extensor tone never shows much recovery, and extensor reflexes, such as the knee-jerk are weak, but sharp and brief. A weak crossed extensor response may accompany a flexor reflex. Owing to the preponderance of flexor over extensor tonus, the lower limbs usually remain semiflexed and hence this condition is often called *paraplegia in flexion*. Flexor response to even a slight stimulus almost anywhere in the paralysed region may ultimately become very widespread and powerful, and becomes the *mass reflex*. This is due to irradiation, and is accompanied by sweating in the paralysed regions and by evacuation of the bladder owing to the rise of intra-abdominal pressure.

During this second stage, reflex micturition and defæcation make their appearance. Gradually also, there appear, at intervals, ' spontaneous ' reflex flexor movements of the lower limbs, which ultimately become almost continual. Hence the muscles do not waste so much as in cases of destruction of the lower motor neurone, *e.g.* that on section of the motor nerves.

* HEAD and RIDDOCH. *Brain*, 1917, **40**, 188.
† FULTON and KELLER. "The Sign of Babinski." 1932 (Thomas, Springfield).
‡ WALSHE *Brain*, 1914, **37**, 269.

In a third, and much later, stage, reflex activity may gradually fail again and then the muscles do waste. Trophic changes such as the formation of ulcers and bed-sores further complicate the patient's existence.

INCOMPLETE TRANSECTION of various degrees, of course, also occurs in man and is frequently distinguished by the fact that extensor tone and extensor reflexes may be enhanced instead of being lost. Flexor reflexes, including the abdominal reflex, on the other hand, are depressed or absent. Hence the condition is often called '*paraplegia in extension.*' The difference is attributed to the section of the cortico-spinal with retention of the vestibulo-spinal tracts, so that the state of reflex activity in the cord more resembles that of the decerebrate than of the spinal animal.

TROPHIC FUNCTIONS OF THE CORD. The reflexes which are excited by nocuous stimuli must be regarded as *prepotent*, in that their inhibitory effect on other reflexes is more marked than that produced by any other quality of stimulus. In the struggle for existence, the reaction to nocuous stimuli must predominate over those due to any other kind, since it is essential for the survival of the animal that these stimuli should be removed or avoided.

It is natural, therefore, that after complete section of the spinal cord, or of the afferent nerves from any part of the surface of the body, there should be a tendency to trophic disturbances such as the formation of ulcers, &c. A spinal patient may easily be burnt by a hot-water bottle. After section of the first division of the Vth nerve, ulceration of the cornea is often produced. These effects are due to the absence of the normal protective reactions of the part, and can be prevented by scrupulous cleanliness and protection of the anæsthetic part from all possible injuries.

There are other trophic effects caused by nerve lesions which cannot be ascribed to the mere absence of protective reflexes. Thus, inflammation of the posterior root ganglia often sets up 'herpes zoster,' or 'shingles,' in the region of cutaneous distribution of the corresponding sensory nerve. Changes in the skin ('glossy skin'), nails, and hair are often seen after irritative injuries of nerves to the part. Section of a motor nerve causes rapid changes in the skeletal muscles supplied, which become smaller, and after months or years may disappear altogether, being replaced by connective tissue.

It seems that the nutrition of a tissue is determined by its activity, and this in turn is under the control of some nerve path. Section of the nerve path, by cutting away the impulses which normally maintain the activity of the part, must at the same time seriously affect its nutrition. Thus the muscles which, though striated, are only under the reflex control of the central nervous system, such as the sphincter ani, only slowly undergo degeneration after section of their nerves, or after extirpation of the lower part of the spinal cord.

THE DECORTICATE PREPARATION. Removal of the entire cerebral cortex gives a preparation that in many respects resembles a normal animal.* In dogs and cats, most bodily functions and also temperature regulation, locomotion and posture are normal, and *righting reflexes* are present, *i.e.* if the animal is put into an abnormal position, it rights itself. There is no extensor rigidity so long as a normal position is maintained, though if held up by the neck an extensor hypertonus resembling decerebrate rigidity makes its appearance. If sections of the brain are made coronally from before backwards, no sign of decerebrate rigidity appears until section reaches a plane passing through or just behind the posterior part of the

* Goltz. *Pflüger's Arch.*, 1892, **51**, 570.

thalamus,* thus indicating that decerebrate rigidity is due to release of lower postural centres (Deiter's nucleus), from the control of centres lying anterior to the pons. Posture and righting reflexes are, in fact, retained almost normally if section passes anywhere anterior to the mid-brain, so that righting reflexes are probably controlled from the mid-brain.

If only the cerebral *cortex* is removed, leaving behind the basal ganglia, the animal may retain many reflexes connected with special senses; thus it will make attempts to eat articles that smell like food, moves to sounds, shows constriction of the pupil of the eye to light, &c. All of these reactions, however normal in appearance, are reflexes mediated by subcortical centres and have no connection with any actual sensation, since the animal is totally deaf, blind and unconscious.

Still more remarkable are the appearances of the expression of primitive emotions, such as rage and fear. A decorticate dog or cat, if disturbed or stimulated in almost any way, however slightly, will exhibit all the signs of violent and exaggerated rage, biting, struggling, lashing of tail, erection of hairs, high pulse-rate and blood pressure, &c. These reactions, though complex and apparently purposive, are also reflex in nature, and are not associated with any state of consciousness on the part of the animal. They are accompanied by intense outbursts of sympathetic activity. These subconscious manifestations are probably due to activity in the posterior part of the hypothalamus,† and are release phenomena, due to the removal of that cortical control which regulates and rationalizes the behaviour of the whole animal. It is probable that unreasoning fear or rage, and emotional instability, in man, are often associated with release of the hypothalamic mechanisms from normal cortical control.

In the higher apes (and we may suppose that it would be similar in man), decortication leads to more profound and evident losses of function than is the case with lower mammals. Normal locomotion is lost and the animal lies on its side in an abnormal posture, with the undermost limbs extended and the uppermost ones flexed.‡

* Thiele. *J. Physiol.*, 1905, **32**, 358.
† Bard. *Amer. J. Physiol.*, 1928, **84**, 490.
‡ Bieber and Fulton. *Arch Neurol. Psychiat.*, 1938, **39**, 435.

CHAPTER XVI

THE EXCITABILITY OF NEURONES AND THE PROPERTIES OF CONDUCTION AT SYNAPSES

CONDUCTION in a reflex arc differs from that in peripheral nerves in that part of it takes place through grey matter, and hence we must look to the properties of the bodies of the nerve cell (soma) or to properties of the synapse for an explanation of the differences between nerve fibre conduction and reflex arc conduction.

It is implicit in the neurone hypothesis that the impulse which emerges by the efferent fibre of a reflex arc is not the same impulse as that which enters the spinal cord by the afferent fibre: it is a new impulse generated by the cell of which the efferent fibre is the axon. Apart from the important evidences of histology, we believe that this is so because (*a*) the time that elapses between the entry of the afferent impulse and the emergence of the efferent response is longer than would be needed for direct transmission, and (*b*) the emergent impulses in response to a given train of afferent impulses, though they may, and often do, tally impulse for impulse, with the number of ingoing impulses, do not necessarily do so; either more or fewer may emerge.

Present-day conceptions of the mechanism of reflex action are indebted chiefly to the investigations of Sherrington and his pupils, and most of their explanations, derived from the study of spinal reflexes, have received general acceptance. It is also generally agreed that an isolated and simple reflex, as diagrammatically represented in Fig. 132, is nothing more than a convenient fiction. When we come to study actual reflexes, we find them to be less simple, and hence interpretation is a matter for detailed analysis. The essential correctness of the conclusions reached by the Sherrington school is shown by the fact that their conceptions have received important confirmation and extension from recent work, which, being more definitely directed to the study of the properties of synapses and neurones, will be outlined first.

TRANSMISSION IN SYMPATHETIC GANGLIA

Synaptic transmission is reduced to its simplest terms in the sympathetic ganglia, *e.g.* the superior cervical ganglion. The fibres which enter from below (pre-ganglionic fibres) have synapses in the ganglion, from the cells of which axons pass up (post-ganglionic fibres) in the neck and are distributed to various structures in the head. One of these structures in the cat is the plain muscle of the nictitating membrane (Fig. 148) and it is the neurones supplying this which have been especially studied. There are at least three other types of neurones in the ganglion, but for the sake of simplicity we shall only consider this one.

When a volley of impulses is started in the pre-ganglionic fibres, by stimulation at *A* (Fig. 148), a volley emerges along the post-ganglionic fibres

and can be recorded from electrodes one of which is placed at *E*.* Also the response can be registered by recording the contraction of the nictitating membrane.† The neurones respond to each afferent impulse with one efferent nerve impulse, and one only.‡ In conduction across the synapse about 3 msec. is lost (= synaptic delay). If the post-ganglionic nerve be stimulated, *e.g.* at B (antidromic stimulation), no impulse can be detected in the pre-ganglionic fibre, *e.g.* at electrode *C*, *i.e.* the synapse will not conduct backwards; but a disturbance is detected at the ganglion itself, showing that the antidromic impulse extends back as far as the nerve cell, and probably into any dendrites it may have. The backfiring of an anti-dromic impulse does not leave the nerve cells unaffected, but is followed by a refractory period, during which a normally arriving preganglionic volley produces no effect.

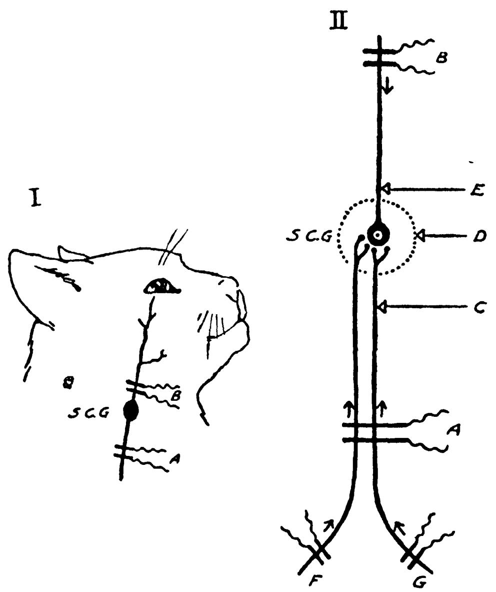

FIG. 148 I, Arrangement for stimulating the cervical sympathetic nerve, preganglionically at A, or post-ganglionically at B II, Scheme showing neurones concerned *A*, *B*, *F*, *G*, points for stimulation *C*, *D*, *E*, points for leading off potential changes. *S.C.G.*, Superior cervical ganglion

When the pre-ganglionic fibres are stimulated, and the action potentials thereby produced are studied in the ganglion and in the adjacent post-ganglionic fibres, these are found to be unlike those which pass along the pre-ganglionic fibre. The changes in the ganglion show three waves, viz. a spike (*S*), a negative after-potential (*N*), and a positive after-potential (*P*) (Fig. 149), and it was shown by Eccles § that these are related to the excitatory process in the nerve cells, in much the same manner as the spike and after-potentials of nerve fibres are related to its excitatory properties.

Immediately following the arrival of the pre-ganglionic nerve impulses at their synapses is a *synaptic delay* of about 3 msec. The spike potential of the soma then occurs if the nerve cell is to discharge an impulse along its axon. During the spike, which lasts about 5 msec., the cell is in an absolute or relative *refractory state*, *i.e.* a second impulse does not excite, or excites less readily.

The after-potentials, according to Eccles, consist of two simultaneous and opposing changes, one tending to make the ganglion (cells) negative and one positive. At first the negative wave is the greater, but the positive one outlasts it (Fig. 149). In the algebraic resultant therefore we have first a negative potential of varying size (*N*) followed by a positive one (*P*). Both

* BISHOP and HEINBECKER. *Amer. J. Physiol.*, 1932, **100**, 518.

† BROWN. *J. Physiol.*, 1934, **81**, 228.

‡ BRONK, TOWER, SOLANDT and LARRABEE. *Amer. J. Physiol*, 1938, **122**, 1.

§ ECCLES. *J. Physiol.*, 1935, **85**, 179, 207, 464; 1937, **91**, 1.

potentials increase in size as the stimulus is increased (Fig. 149). During the prevalence of the N-wave the ganglion cells are in a state of supernormal excitability, in consequence of which they respond to a smaller second

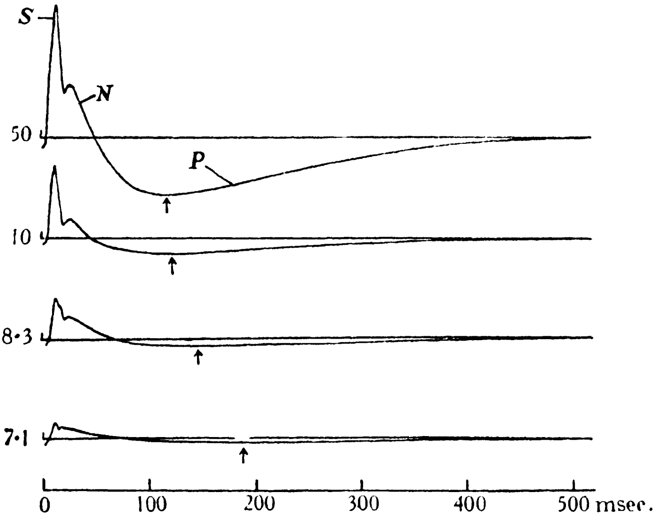

FIG 149 Ganglionic Action Potentials in superior Cervical Ganglion set up by Preganglionic Stimuli whose strengths are shown at left Arrows mark the points of maximum positivity *S*, *N*, and *P* are respectively, spike, negative and positive waves (ECCLES *J Physiol*, 1935, **85**, 464)

stimulus and with shorter synaptic delay than before (temporal summation). This stage may last for 150 msec. An N-wave always accompanies the application of a stimulus to the nerve cells, *e.g* at the synapse, even if this is too weak to excite them (Fig 149). The appearance of the N-wave coincides

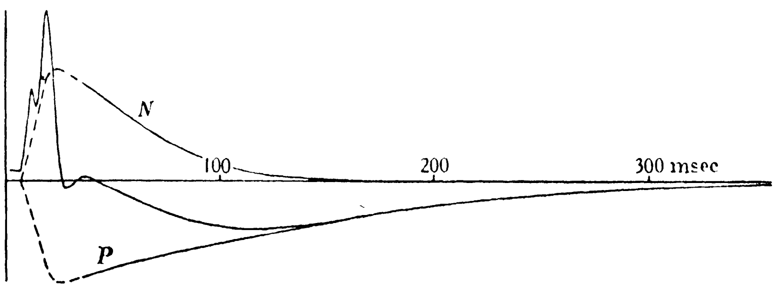

FIG 150 Ganglion Potential Curve, and the Reconstructed Negative (N) and Positive Waves, which sum algebraically with the spike to give the complete curve. (ECCLES. *J Physiol*, 1935, **85**)

with the condition of the cell which was called by Eccles and Sherrington the *central excitatory state* (c.e.s.), *i.e.* a proneness to excitation. Hence if two subthreshold stimuli are so spaced in time that the second arrives during the c.e.s. produced by the first, the raised c.e.s. may enable the cell to respond and discharge an impulse. When the c.e.s. reaches a certain level the cell discharges its impulse at the slightest provocation, and when it has done so this hastens the subsidence of the c.e.s.

Another kind of summation may be illustrated with the superior cervical ganglion. If stimuli of subthreshold strength be applied to two branches (of the annulus of Vieussens) at *F* and *G* (Fig. 148 II), excitation of the cells may result. This is called *spatial summation* because the stimuli act at different points on the soma.

The P-wave, which follows the N, is indicative of a corresponding change in the excitability of the cell, which is now reduced—a condition called *central inhibitory state* (c.i.s.). During this phase, which lasts for 500 msec., the cell is less excitable, *i.e.* its threshold of excitability is raised.

The results of backfiring an antidromic impulse from the post-ganglionic fibres into the ganglion show that the neurone is caused to go through processes very similar to those which occur when it is stimulated in a forward direction.* Thus the ganglion shows a spike potential followed by the N and P-waves, the P-wave being very large and prolonged. After the backfiring there is an absolute refractory period of 2 msec. followed by a long lasting state of depressed excitability running parallel with a prolonged P-wave. In other words, the cell has discharged an impulse, though this has been unable to pass backwards through the synapse, and the discharge has been followed by a prolonged central inhibitory state.

The after-potentials are essentially due to changes of polarization in the cell body and do not extend far down the post-ganglionic axons; probably they merely spread electrotonically. They indicate a *state of excitability* of the cell and its synapse and *not* the propagation of a nervous impulse. The N-wave is reduced by nicotine, which also destroys c.e.s.

When the P-effect predominates, a further P-effect is less easily produced than an N-effect; the production of the P is said to be *occluded* and that of N *facilitated*, and *vice versâ*, if the N-effect predominates, there will be occlusion of N and facilitation of P.

In further investigations Eccles † advanced the following explanation for the means by which the preganglionic impulses excite the nerve cells. The arrival of the preganglionic impulse sets up at the synapse a brief excitatory state called the *detonator response*. This induces through a medium called the '*synaptic transmitter*' a localised response at the surface of the cell and when this spreads and summates with the detonator responses from other synaptic terminals and reaches a sufficient level, the cell explosively discharges an impulse. The detonator responses are over within 6 msec., which makes it hard to believe that the synaptic transmitter can be a chemical mechanism such as acetyl choline, as claimed by Brown and Dale.‡ Moreover, the duration of the detonator response is not increased by eserine. Hence Eccles regards the synaptic transmitter as the action potential of the preganglionic impulse. The c.e.s. set up in the cell does not begin until after the synaptic delay and lasts much longer, reaching a maximum at 10 to 15 msec. It can therefore have no direct action in discharging the impulse, but merely increases the 'explosiveness' of the cells.

TRANSMISSION AT MOTONEURONES

We may now consider the phenomena of excitation and transmission in less simple neurone arrangements, such as those found in the central nervous system proper. Most valuable investigations have been made by Lorente de Nó § on the motoneurones of the oculomotor nucleus, which supplies the motor fibres of the third, fourth and sixth cranial nerves.

The oculomotor neurones are a final common path in three reflex arcs, one from the labyrinth, one from the fifth nerve and one from the neck

* Eccles. *J. Physiol.*, 1936, **88**, 1.

† Eccles. *J. Physiol.*, 1937, **91**, 1.

‡ Brown. *Physiol. Rev.*, 1937, **17**, 485.

§ Lorente de Nó. *Amer. J. Physiol.*, 1935, **111**, 272, 283; **113**, 505, 524. *J. Cell. Comp. Physiol.*, 1935, **7**, 47; *J. Neurophysiol.*, 1938, **1**, 187, 195, 207; 1939, **2**, 402.

muscles. The afferent fibres from these pass in or near to the posterior longitudinal bundle which runs near the floor of the fourth ventricle and can be stimulated there.

The arrangement of some of these experiments is illustrated by Fig. 151.

These neurones can be stimulated, either *viâ* synapses which are formed with fibres of the posterior longitudinal bundle, by electrodes in the floor of the fourth ventricle at the point F in Fig. 151, or directly at C_1, by electrodes

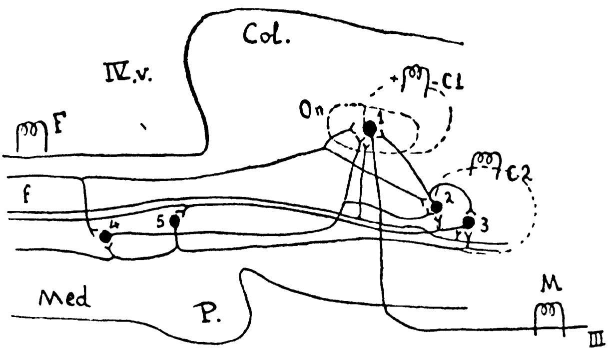

FIG 151. Arrangement for studying the responses of the oculomotor motoneurones IV.v. Fourth ventricle; Col, colliculi, Med, medulla; P, pons; F, C_1, C_2 and M., positions for stimulating electrodes; O n, oculomotor nucleus; III, oculomotor nerve; f, fibres in posterior longitudinal bundle stimulated by F shock; 1, motoneurones of third nucleus; 2, 3, 4 and 5, internuncial neurones of reticular substance; Shocks at C_1 and C_2 stimulate motoneurones and internuncial cells respectively (LORENTE DE NÓ. *J. Cell. Comp. Physiol.*, 1935, **7**, 47.)

buried in the colliculi, and the response can be recorded as an action potential in the third or fourth nerves, or in the eye muscles they supply.

By stimulation at the electrodes M, on the third nerve, the cells (1) of the nucleus receive an antidromic impulse, and, of course, the muscle is stimulated by the forward impulse. The cells and fibres concerned in these experiments are of higher excitability and faster action than those we have just studied in the sympathetic system, but apart from this difference in the time-scale there is a general similarity between the phenomena in the two places.

Direct stimulation of the nerve cells (position C_1) with one shock, gives, with no appreciable latency, a single volley of impulses; two subliminal shocks, if in sufficiently quick succession, also excite by latent addition. For this to occur they must be not more than 0·2 to 0·3 msec. apart. This shows that the excitatory process caused by stimulation of the motoneurone is of the same duration as that of nerve fibres. Having once responded, the motoneurones pass through a *refractory phase*, which is absolute for about 0·5 msec., which again is identical with that for nerve fibres.

The refractory phase is followed by a long period (30 msec. or more) of *subnormal excitability* which resembles that of a nerve fibre that has received a number of stimuli in quick succession (Fig. 106, p. 191). It is doubtful whether there is a supernormal phase resembling that shown by nerve after a single conditioning stimulus.

Thus far, then, there is a close similarity between the properties of the soma and those of the nerve fibre.

Synaptic stimulation of the motoneurones can be brought about by a stimulus applied at the F electrodes (Figs. 151 and 152). When excited

in this way there is a delay of about 0·6 msec. in transmission across the synapse. If the motoneurone does not respond within this time it will not respond at all unless restimulated by a new impulse. Since the major part of the spike potential in a nerve fibre (or synaptic knob) lasts about 0·5 msec., it would seem that it is the action potential at the synaptic knobs which is the synaptic transmitter of excitation.

An antidromic impulse fired into the cells from the point A (Fig. 152) on the motor nerve, discharges the neurones and is followed first by a refractory phase and then by a state of depression (Fig. 152), which lasts for at least 30 msec., just as happens when the cells are stimulated directly; during these stages of depression synaptic stimulation (by an F-shock) produces less or no effect; after repeated A-stimuli the depression lasts still longer.

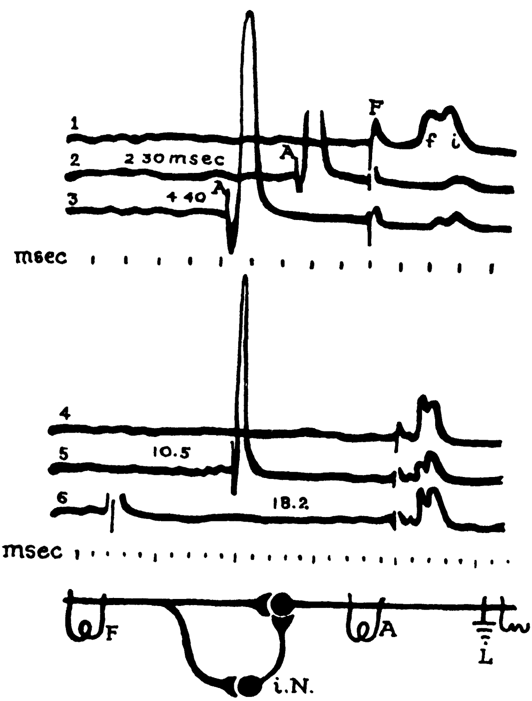

FIG. 152. Discharge of Motoneurones by Antidromic Shock; Subsequent Recovery Oculomotor preparation of rabbit. Scheme showing stimulating electrodes at F and A in lower figure Lead from IVth nerve at L. i N = internuncial neurone. Tracings 1 and 4; response to F shock to afferent fibres consists of two waves, f from the direct path, and i by impulses delayed at the internuncial neurone Moment of shock shown by sharp deflection Tracings 2, 3, 5 and 6, the F shock has been preceded by an antidromic maximal stimulus at A, the intervals between A and F being shown on records. Time scale in msec is quicker for 1, 2 and 3 as shown in scales (Modified from LORENTE DE NÓ and GRAHAM, *Amer J Physiol*, 1938, **123**, 388)

Internuncial Paths. The response to synaptic excitation is often complicated by the existence of alternative paths through internuncial neurones, and as it is probably to such circuits that some of the characters of conduction through reflex arcs are due, we must briefly consider these. Elementary reflex arcs such as those depicted in Figs. 132 and 133 are merely convenient abstractions, and are probably quite rarely operative. Many neurologists believe, with Ranson and Hinsey,* that in addition to neurone relays intercalated on a direct path there are often multiple by-path internuncials (*principle of plurality of connections*) and also reverberatory side arcs or 're-exciting circuits' (*principle of reciprocity of connections*).† These important types of connections are illustrated in Figs. 153 and 168.

It is clear that circuits of these two types introduce new possibilities into

* RANSON and HINSEY. *Amer. J. Physiol.*, 1930, **94**, 471.
† LORENTE DE NÓ. *Arch. Neurol. Psych*, 1933, **30**, 245.

the phenomena of conduction in the central nervous system, because each synapse causes a delay of about 0·6 msec. As a result of the principle of multiple connections, an afferent impulse, in addition to being conducted direct to a terminal point, may also be delivered by delaying paths to the same or other points and at later times, so that the excitation is spread out both spatially and in time (see Fig. 152). In consequence of the closed chain one impulse, or an afferent train of only a few impulses, can be worked up by re-excitation through delaying circuits into a longer train of impulses. With both types of circuit it should be noted that there results a more prolonged repetitive discharge of impulses into one or several points of a neurone pool.

Facilitation. The neurones of the oculomotor nuclei are most responsive when their c.e.s. is kept up by continual bombardment from internuncial neurones, too weak to actually excite the cells to discharge. Such cells are in a state of what is called by Sherrington '*subliminal excitation.*' Each cell in this condition is ready, on receipt of a small additional stimulus, to discharge an impulse. This additional stimulus cannot come in the form of a second impulse at the same synapse, because the refractory period of the fibre is longer than the period of latent addition in the cell (see below).

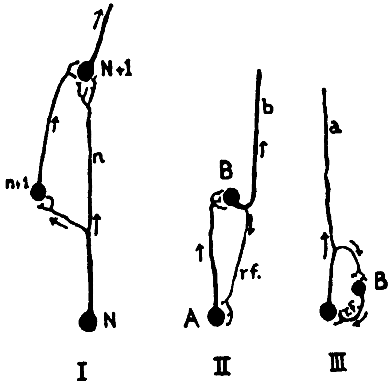

FIG 153. Diagrams illustrating connections within chains of neurones. I illustrates 'law of plurality of connections,' II and III the 'law of reciprocity of connections.' Arrows show directions of impulses. (LORENTE DE NÓ. *Arch. Neurol. Psych*, 1933, **30**, 245.)

Now if we consider the arrangement of the synaptic knobs in relation to

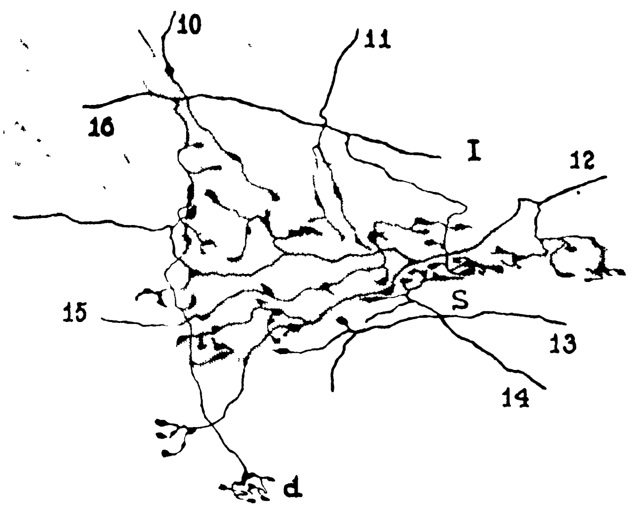

FIG. 154. Synapses on a large Internuncial Cell of the Spinal Cord of a Kitten. (LORENTE DE NÓ. *J. Neurophysiol.*, 1938, **1**, 200.)

the soma of a nerve cell, as shown in Fig. 154, it will be seen that a neurone receives contributions from several axon terminations, and that the synaptic knobs (of which there may be 1,000 or more on a single soma), are not

necessarily terminal, but are distributed along the course of the fibrils in interlacing clusters of knobs surrounding the soma and dendrites, the synaptic terminals of different fibres often being at a distance from one another.

It would seem probable, then, that facilitation would be seen when the later impulses arrived at new loci on the same soma. Evidence was, in fact, obtained by Lorente de Nó that usually more than one afferent fibre needs to be stimulated in order to activate, in any given zone of the soma, a sufficient number of synaptic knobs to cause excitation culminating in the discharge of a nerve impulse. A similar conclusion was reached by Sherrington for the motoneurones of the spinal cord. If too limited a zone at the surface of the neurone is excited the excitation remains a local non-propagating one which presumably dies down unless reinforced within a brief period (0·2 msec.), just as occurs with the non-propagated disturbances in nerve fibres.

In consequence of this it often happens that the stimulation of motoneurones by one set of synapses may be too weak and localized to result in excitation. Many of the motoneurones of the oculomotor nucleus are normally in a state of subliminal excitation as a result of the receipt of impulses from internuncial neurones, *e.g.* those from the labyrinth, and under these circumstances a stimulus applied at F in Fig. 151 is able to excite them.

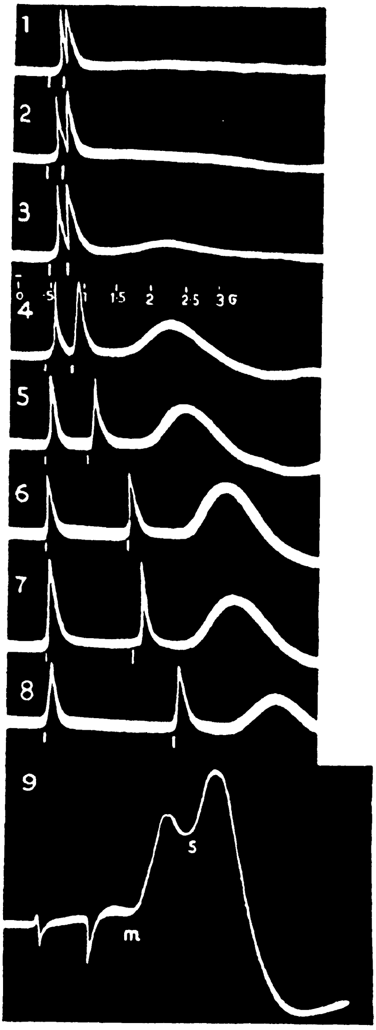

FIG. 155 Facilitation in motoneurones *Curves* 1—8. An F-shock, followed at the interval shown, by a C-shock. No effect at intervals below 0 25 msec., maximal effect at 1·25 msec. *Curve* 9: Two C-shocks, the first one sub-threshold, the second one adequate, 1 msec. later The m-wave the direct motor response, the s-wave the delayed synaptic response. (LORENTE DE NÓ. *J Cell Comp. Physiol.*, 1935, 7, 47)

This 'tonic' subliminal excitation is abolished by destruction of the reticular substance of the medulla and pons, in which the internuncial paths lie. When this has been done a single shock at F gives no response, though two shocks 1 to 1·5 msec. apart usually do. Similarly, if a subliminal electrical stimulus is given to the motoneurones at the point C_1 (Fig. 151), and followed by a subliminal stimulus *viâ* the synapse by stimulating at F, say, 1 msec. later, a response may ensue.

At first sight the fact that facilitation occurs best when F shocks are as much as 1 msec. to 1·5 msec. apart seems to conflict with the statement made above that the maximum period of latent addition of subliminal stimuli is 0·2 to 0·3 msec., after which

the local c.e.s. dies away. The explanation shows that the matter is less simple than appears at first sight, and is as follows: The F shock causes local synaptic excitation, and this dies away in 0·2 msec.; but it also excites a by-pass neurone having its own synapse, *viâ* which a delayed impulse reaches the motoneurone about 0·6 msec. later; if there are two by-pass neurones in series the delay will be at least 1·2 msec., and if at this moment, or within a short time after it, a second impulse arrives by the direct fibre from F, latent addition will occur. Facilitation, according to this, is therefore really due to the arrival, by two different paths, of almost simultaneous impulses at different synaptic points on the same soma. If in addition to these by-pass circuits we have closed reverberatory circuits the bombardment of the motoneurones can be kept up for much longer periods, so that facilitation may still be possible 30 msec. or more after a first stimulus.

This phenomenon of facilitation is well shown by Fig. 155, in which a subliminal F-shock is followed at varying intervals by a subliminal C-shock, *i.e.*, by a direct shock to the motoneurons. It will be seen that the earliest indication of a response was when the interval was something over 0·25 msec. Up till then the time is occupied by transmission from the F electrodes, say, 0·1 msec., followed by a lowering of the threshold of the motoneurone which reaches its maximum after about another 0·15 msec. After this we may suppose that delayed-path impulses continue to arrive at the motoneurones, and reach a maximum at 1·25 msec., afterwards declining. The effect of the C-shock is therefore greatest when it follows the F-shock at about 1·25 msec. (curve 7, Fig. 155).

It often happens that the latent period of the response of a motoneurone is considerably reduced by a previous excitation. This is not because facilitation shortens the synaptic delay, which is almost unaltered; it is because facilitation enables excitatory impulses to be effective directly without further (delayed) reinforcement through internuncial neurones.

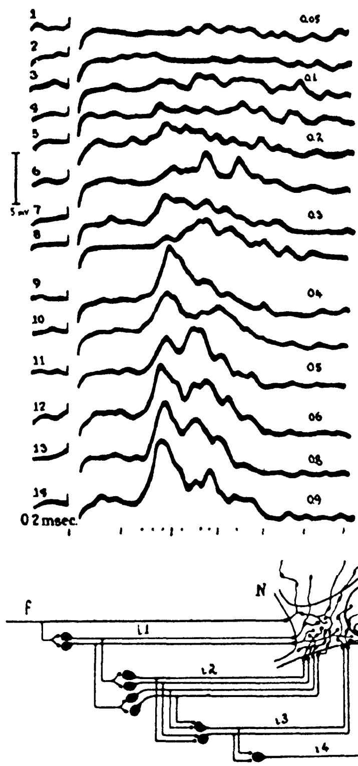

FIG. 156 Responses of Oculomotor Neurones recorded from IVth Nerve to F-shocks of strengths shown on right. Diagram below illustrates the temporal and spatial distribution of impulses arriving at the motoneurone N, after a shock at f. i_1–i_4 are internuncial by-paths. Each synapse means a delay of 0 6 msec. Note that each fibre has several synaptic knobs on the soma. (LORENTE DE NÓ. *J. Neurophysiol*, 1938, 1, 207).

It would seem that *the multiple chain of neurones is the normal unit of transmission in the nervous system*, and supersedes the classical and diagrammatic reflex arc of a fixed number of synapses. If the number of neurones in use in the chain at any time is large, facilitation may be continuous, and any applied stimulus may be followed by an '*after-discharge*.' As is shown in Fig. 156, in which there was a background of internuncial bombardment, an increase in the strength of an F stimulus leads to a more sustained response, with shorter latent period. This response, however, even with the strongest stimuli, never engaged more than about a third of the possible motoneurones. As the Oxford school would say, the motoneurone pool response was *fractionated* into an active and an inactive group, the latter, or part of it, representing a *subliminal fringe*.

Inhibition. Sometimes, after a strong conditioning F-shock, there is a brief period of facilitation followed by a period in which responses to the testing shock are reduced or abolished. The explanation * of this phenomenon is that a refractory state and a state of subnormal excitability, as we have seen, follows the excitation of a neurone. Hence, since the first stimulus was a strong one, many internuncial neurones became involved—hence the early facilitation. But after this those neurones became refractory, and so the second shock from F received no facilitation and became ineffective. *Inhibition, therefore, is not an active process happening in the neurones, but is merely lack of facilitation.*

In some parts of the central nervous system inhibition is very long lasting. Thus in the cerebral cortex a conditioning shock leads to prolonged extinction of the response to a testing shock.†

TRANSMISSION IN THE SPINAL CORD

The phenomena of transmission in the spinal cord are necessarily more complex than those we have so far considered, because there are many possible paths.

When a single volley of impulses is fired along an afferent nerve, *e.g.* by giving a weak single shock to the central end of the popliteal nerve in a spinal cat, a reflex twitch of the ipsilateral flexor muscles, *e.g.* tibialis anticus, results. If a record of the electrical potential changes in the muscle (or in the motor nerve) is made, it is found that, if the afferent shock was weak, one efferent volley only is caused, *i.e.* the efferent fibres, or some of them, have each conducted one impulse in response to one afferent impulse.‡

If a very strong stimulus is given to the afferent nerve, then two impulses may pass along it, two also emerge by the motor path, and the muscular contraction is longer because of summation. But it also happens that a strong stimulus may cause only one afferent impulse, but two or more efferent ones, with a resultant longer muscular response. This must mean that in some manner the motoneurones have been caused to fire at more than one moment in response to one afferent volley—a result called *repetitive discharge.* This phenomenon is readily explicable in the light of the information given in previous pages and does not necessarily mean that all the individual neurones are firing repetitively. The stronger stimulus excites more afferent fibres and causes impulses not only along the more direct paths but also along new internuncial paths, by which a belated volley of impulses arrives at the motoneurone pool, and excites the same or a fresh set of motoneurones, so that a later volley of impulses is fired either along the same efferent fibres, or along new ones not previously employed.

When two weak stimuli are given in succession to an afferent nerve for the spinal flexor arc, two impulses emerge from the efferent side if the two stimuli are more than 1·0 to 1·9 msec. apart.§ If the interval is less than this, the second stimulus is ineffective because it falls in the refractory period of some part of the reflex arc. Actually this refractory period (absolute and relative) may be as much as 4 to 6 msec., but when two or more afferent stimuli are given at, say, 1 msec. intervals, the second one travelling in the wake of the first is conducted more slowly, so that it only arrives

* LORENTE DE NÓ. Symposium dedicated to Professor Beritoff Academy of Sciences of U.S.S.R. Georgian Branch, Tiflis, 1936, 231.

† DUSSER DE BARENNE and MCCULLOCH. *Proc. Soc. exp. Biol. Med.*, 1934, **32**, 529.

‡ ADRIAN and FORBES. *J Physiol*, 1922, **56**, 301.

§ ADRIAN and OLMSTED. *J. Physiol.*, 1922, **56**, 426.

at the centre about 6 msec. after the first, *i.e.* not until the arc has recovered.

It cannot be said precisely where in the arc the refractory state is longest. There is, of course, a refractory state in the nerve, but the absolute refractory period is only of the order of 0·4 msec. and the relative about 3 msec. for the fast fibres. It would seem probable that the neurones of the spinal cord have very similar properties, and that owing to the delayed conductance for second impulses these do not reach the neurones until their refractory period has passed off.

If a series of stimuli are given to the afferent nerve, so that a train of impulses passes along it, the efferent nerve also conveys a train of impulses at the same frequency, provided the afferent stimuli are not too close together. The minimum interval between stimuli in a series is about 3·5 msec., *i.e.* the maximum frequency at which a series of afferent nerve stimuli can be translated into efferent ones is about 300 per second.* It would appear then that if, in these experiments, the same motoneurones respond to each volley, these motoneurones cannot be made to send out a regular series of impulses at a greater frequency than about 300/sec. (and if the spinal cord is cooled the rate is slower). The muscle, as we already know, is able to respond to stimuli faster than this, so that there is no question of the motoneurones being able to discharge impulses more rapidly than the muscles can follow them.

In reflex contractions brought about by ordinary sensory stimuli, *e.g.* pinching the foot, the impulses pass along the motor nerves at a frequency up to 100 to 250/sec. With frequencies of this order the muscle, of course, gives a perfectly smooth contraction, and the component excitations can only be seen in the electrical record.

Cord Potentials. Studies of the electrical potentials of the cord yield further important information. If an afferent volley is sent into a dorsal root, it may be traced as a spike potential into the cord and for some distance

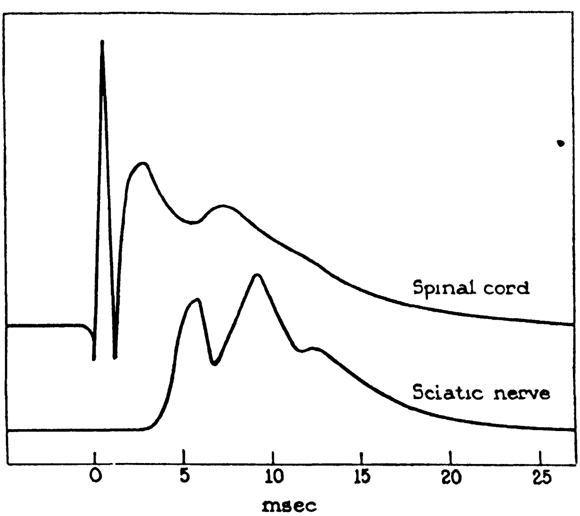

FIG. 157. Relation of Afferent Spike and Negative Internuncial Potentials in the Lumbar Cord of the Spinal Cat to the Reflex Discharge into the Sciatic Nerve. The positive after-potentials come later and are not shown. GASSER. Harvey Lectures, 1936, 182.

* COOPER and ADRIAN. *J. Physiol*, 1924, **59**, 61.

along the dorsal columns. Leads from the spinal cord surface show that this spike potential is followed by slow waves, which according to Gasser are due to the excitation of internuncial neurones.* The potentials produced in the motoneurones are obscured by these, but their discharge is indicated by the volley which finally emerges from the ventral root. The original afferent spike, the discharge potentials of the internuncials, and the efferent discharge along the motor nerve are shown in Fig. 157. It will be seen that the discharge along the efferent nerve runs parallel with the curve of negative cord potential. This negative phase is attributed to the excitation of internuncials and not of motoneurones, because an antidromic impulse which would discharge the latter, does not modify it. During the time that there is a negative potential (10 to 20 msec.) the internuncial neurones are emitting impulses which pass on to the motoneurones. According to Hughes and Gasser † the negative cord potential represents the overlapping of these internuncial spikes. During this period the reflex arc is facilitated. Each spike discharge of an internuncial is presumably accompanied by a refractory period after which there is no demonstrable negative after-potential comparable with that of fresh nerve, and accordingly there is no supernormal phase like there is in fresh nerve fibre, but instead, there is an immediate subnormal state, accompanied by prolonged (100 msec. or more) positive after-potential (Fig. 158), as is seen in nerve fibres after many previous stimuli (v. p. 106, Fig. 191). During this period the reflex arc is therefore in a state of depressed excitability, so that a second volley, say 50 msec. after the first causes a smaller response, *i.e.* the reflex is *inhibited*.

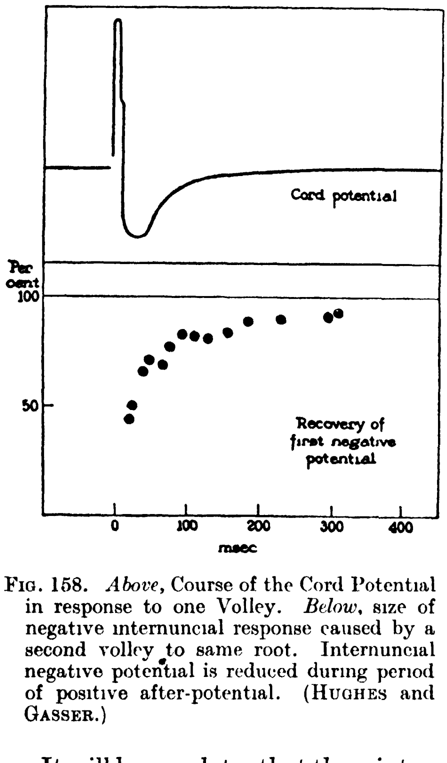

FIG. 158. *Above*, Course of the Cord Potential in response to one Volley. *Below*, size of negative internuncial response caused by a second volley to same root. Internuncial negative potential is reduced during period of positive after-potential. (HUGHES and GASSER.)

It will be seen later that these internuncial waves negative (10 to 20 msec.) and positive (70 to 200 msec.) correspond roughly with the time course of c.e.s. and c.i.s. respectively.

Root Potentials. A somewhat different interpretation of spinal cord potentials is given by Barron and Matthews,‡ who recorded the potential changes in the dorsal and ventral nerve roots, when afferent impulses entered the cord as a result of either electrical stimuli to the dorsal roots or natural stimuli to the nerve terminals. In both cases they found that (apart from spike potentials), slow negative potential waves occurred, *i.e.* the electrode nearer the cord was negative to the other. The waves last for from 0·1 sec. up to periods of seconds, and are seen in both dorsal and ventral roots

* GASSER and GRAHAM. *Amer. J. Physiol.*, 1933, **103**, 303.
† HUGHES and GASSER. *Amer. J. Physiol.*, 1934, **108**, 295, 307.
‡ BARRON and MATTHEWS. *J. Physiol.*, 1938, **92**, 276.

whenever these are involved in reflex actions (Fig. 159). The waves in the dorsal roots are seen not only in stimulated roots, but also in neighbouring roots on the same or the opposite side. Hence they cannot be caused by the passage of nerve impulses along all the nerve fibres that show the waves. The spike potentials, when they occur, of course, represent the passage of nervous impulses, and need not be further described. They are always superimposed on the slow negative waves, and these are larger and longer

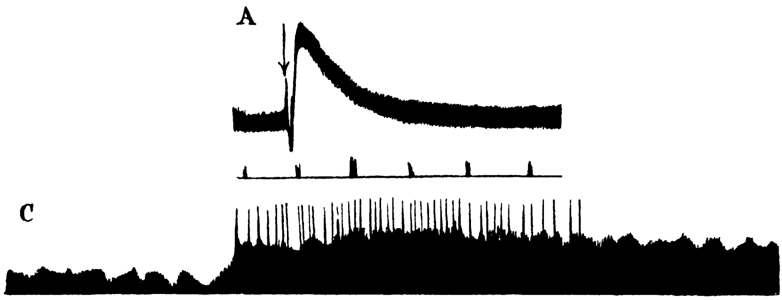

Fig. 159. A. Potential Changes in Dorsal Rootlet of Frog when Stimulated by Single Shock. Spike followed by slow potential (Time — 0·1 and 0·02 sec. intervals.) C. Potential changes in ventral root of L_6 in cat (fibres reduced by partial section). Stimulus by squeezing toes. The shower of spikes lasts 0·7 sec. and is superposed on a negative potential wave lasting beyond it. (Barron and Matthews. *J. Physiol.*, 1938, **92**, 276.)

the more intense and prolonged the afferent stimulus. With very brief and weak stimuli the slow potentials may occur in the dorsal roots without any spikes appearing at all. What they indicate is a tendency to discharge spikes. These slow negative potentials, which are probably similar to those ascribed by Hughes and Gasser to overlapping internuncial spikes, are differently interpreted by Barron and Matthews. They regard them as

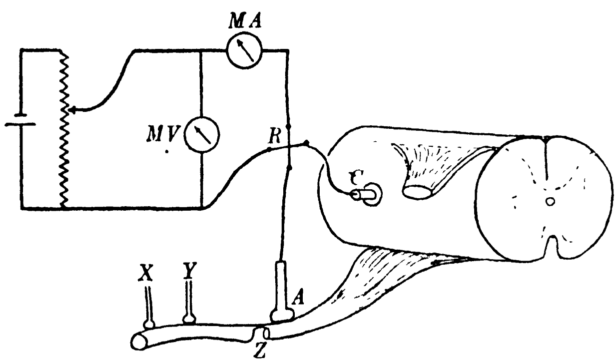

Fig. 160. Depolarization of Motoneurones by Constant Current. *A*, anode; *C*, cathode; *X*, *Y*, recording electrodes; *Z*, point at which all active fibres but one have been divided. (Barron and Matthews. *J. Physiol.*, 1938, **92**.)

being due to a depolarization of the axons of the roots due to electrotonic spread from sites where an active depolarization obtains. The roots merely act as leads by which electrotonic currents can be tapped off from these sites (*v.* p. 202). This state of depolarization is an essential accompaniment of excitation, and in fact is the ultimate cause of it. When a motoneurone is sufficiently depolarized it emits nerve impulses rhythmically at a frequency depending, among other things, on the degree of the depolarization. In

evidence of this they adduced the fact that it is possible by the passage of a *constant* current to the surface of the cord (Fig. 160) to depolarize the motoneurones and so to cause them to discharge rhythmically (Fig. 161). In this respect the motoneurones are therefore different from normal nerve fibres and more closely resemble the sensory terminals.

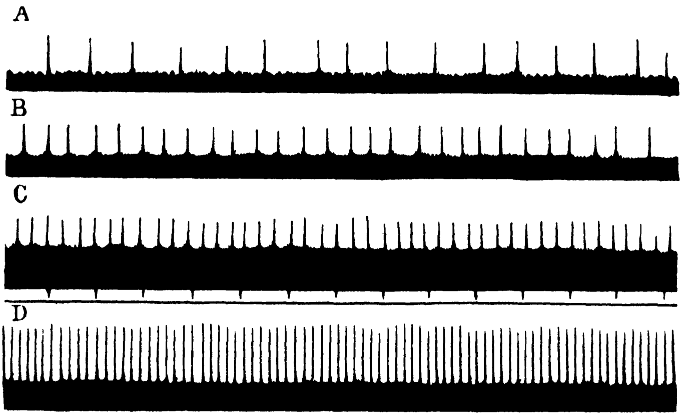

FIG. 161. Stimulation of Single Motoneurone of Frog by Constant Current of Strength increasing from A to D. (BARRON and MATTHEWS. *J. Physiol*, 1938, **92**)

Owing to electrotonic spread, when the ventral root is led off the record indicates not merely the discharge of impulses, but, for a short distance along the root, also the depolarization of the motoneurones which causes it. When the dorsal root is tapped the depolarization at the fine non-medullated feltwork in which the afferent fibres end also spreads electrotonically for a short distance along the root. The relation of these depolarizations to the transmission of impulses is indicated in Fig. 162.

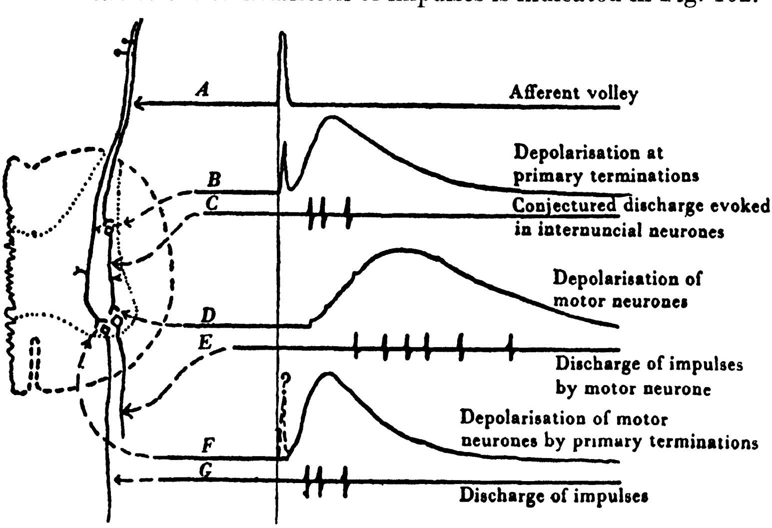

FIG. 162. Diagram summarizing the Observations and Deductions of Barron and Matthews. (BARRON and MATTHEWS. *J. Physiol.*, 1938, **92**, 316.)

For Reference.

GASSER *et al* (1939). Symposium on the Synapse. (From *J. Neurophysiol*, 1939, **2**, 361.) (Baltimore: Thomas.)

CHAPTER XVII

CHARACTERISTICS OF REFLEX ACTIONS

In his classical "Integrative Action of the Nervous System," Sherrington showed in 1906 how reflex actions are organized and modified to serve the needs of the individual, and the continued researches of his school from that time to the present have further widened and deepened our knowledge of the phenomena of nervous action. Most investigations on reflex action have been on motor reflexes, because of the ease with which they can be studied quantitatively. A convenient measure of a response in a given muscle is the isometric tension produced when it contracts. We may consider a given muscle to be innervated from a group of ventral horn nerve cells, which is called the *motoneurone pool*.

The Motounit. Each motoneurone has an axon which branches freely near its termination and so supplies many muscle fibres. These all contract together. The actual number can be determined by dividing the number of muscle fibres in a given muscle by the number of nerve fibres in its motor nerve. The average is around 150 fibres per motoneurone, but is greater with powerful muscles (*e.g.* 500 in cat's gastrocnemius) and less with delicate and precise ones such as the eye muscles. A motor neurone with all its muscle fibres is called a *motor unit* or motounit. If the motor nerve to a muscle is given a maximal tetanic stimulation for a second or so, the tension produced when divided by the number of motounits, gives us the maximal tension per unit. This for the cat varies from 6 g. for semitendinosus to 30 g. for gastrocnemius ; for simple twitches tensions about one-fourth as great result. A muscle like the cat's soleus having about 250 motor units and giving a maximal tension of about 2500 g. can thus have 250 gradations of 10 g. each as its tension rises from zero to maximum.

CHARACTERISTICS OF REFLEXES. Let us first take as an example the flexor reflex already mentioned (p. 243) ; it illustrates certain properties common to them all.*

(1) *Localization.* The flexor reflex is caused by a nociceptive stimulus applied to the same limb ; the response is quite regular and automatic in appearance. It is determined by the fact that the nerve fibres, which carry the impulses from, say, the toe, into the spinal cord, divide there and make connections with the motoneurone pools whose axons are distributed to the several muscles involved in the reaction. The connection of the sensory with the motor neurone may not be direct, since in most cases the impulse has to pass through intermediate neurones before arriving at the motor neurones. The path of the impulse, however, is definite and unvarying, despite the fact that innumerable other paths are theoretically possible.

(2) *Co-ordination.* In the flexor response all the physiological flexors are brought into action. A reflex achieves not the contraction of a particular muscle, but the performance of a significant movement. This always involves *groups of synergistic muscles.*

The effect varies with the strength of the stimulus. The minimal effective stimulus causes simply flexion of the foot. As its strength is increased, this

* Eccles and Sherrington. *Proc. R S.*, 1931, **107B**, 511, 535, 586, 597.

is attended by flexion of the leg on the thigh, and finally by flexion of the thigh on the body. With still stronger stimulus there is a spread to the opposite hind limb, which, however, extends. Increase in the strength of stimulus causes not only an increase in the strength of contraction of the reacting muscles, but also a spreading of the reaction to more and more muscles, or groups of muscles. The spread occurs always in definite order. The stimulus when represented by the prick of a needle can affect only one or two nerve fibres. The impulse carried along these spreads in the cord, reaches the motor neurones and causes them to discharge. The first discharge is, as a rule, limited to those in the immediate proximity of the entering impulses, but, even when minimal, involves the simultaneous action of more than one ventral root. We may say that the response is determined to a certain extent by the proximity of the afferent to the efferent paths, but that it is nearly always plurisegmental, the most important determining factor being the adaptation of the movement to the stimulus.

The gradual spread of the response with increasing strength of stimulus is spoken of as '*irradiation*.' The nature of the response is determined by the place of application of the stimulus, and by the quality of the latter. While a painful stimulus causes flexion of the leg, firm pressure on the plantar surface of the paw causes the extensor thrust—the 'stepping' reflex. However extensive the irradiation, the muscles which are set into action are always such that their actions co-operate towards a given end. Thus, when the impulse spreads to the opposite limb and produces an extension, the reaction is such as would ensue when the animal steps on a sharp point and immediately retracts the irritated limb away from the injurious agent, while it extends the other limb in the first act of movement away from the spot.

(3) *Reciprocal Innervation.* A superficial study of this reflex might lead us to suppose that, by the varying resistance in different synapses, the impulses are directed so as to affect exclusively the muscles whose contraction will co-operate in the primary reflex. Such a description, however, would represent only one-half of the process. Every muscle in the body is in a variable state of tone. If the tone in the opponent muscles is not to interfere with the carrying out of a reflex movement, there must be some means by which it can be inhibited. Such an inhibition always occurs, and the remarkable fact was brought out by Sherrington that the impulses, which start on the surface of the body and set loose the co-ordinated contraction of certain muscles, spread at the same time to the motor mechanisms governing the muscles antagonistic to the movement, and exercise on these an inhibitory effect.

This inhibition can be best shown in the following way: The extensor thigh muscles are cut away from their attachments to the tibia, and the patellar tendon is connected with a recording lever. The flexor muscles of the knee are similarly detached from their insertions and connected with another lever. On then exciting the flexor reflex by stimulation of an afferent nerve of the leg, the lever attached to the flexors shows a contraction, while that connected with the patellar tendon falls (Fig. 163), showing that the extensor muscles have undergone actual elongation. The inhibition of the extensor tone is thus a direct result of the primary stimulus.

(4) *Delay.* When the latent period that elapses between stimulus and response in the flexor reflex is measured, and the time taken in conduction along the nerve fibres is deducted, we get what is called the *central reflex time.* Thus if the latent period was 10·4 msec., and the nerve conduction time was 6·5 msec., then the time spent in central processes was 3·9 msec.

A.

B.

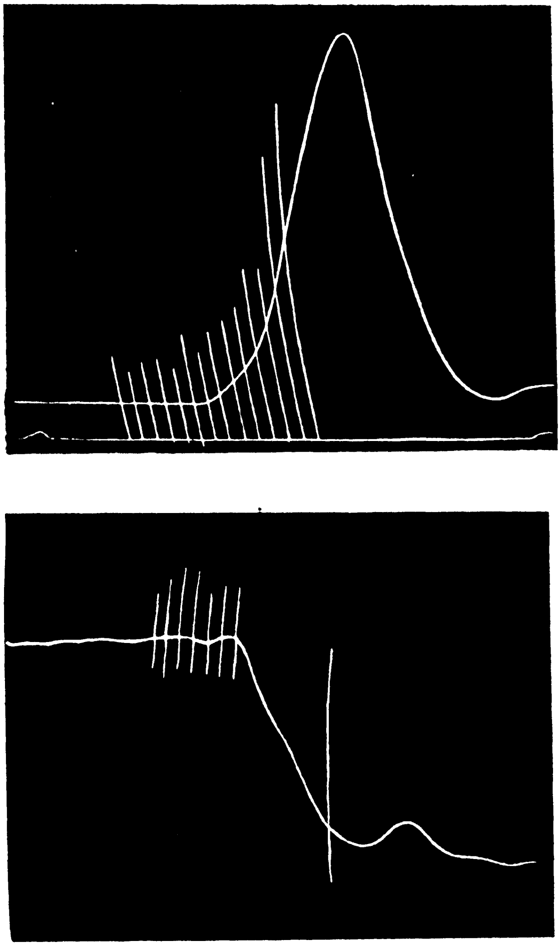

FIG. 163, A and B. (SHERRINGTON.)

The Flexion Reflex observed as Reflex Contraction (Excitation) of the Flexor Muscles of the Knee (A), and as Reflex Relaxation (Inhibition) of the Extensor Muscle (B).

The stimulus was a series of weak break induction shocks applied to a twig of the internal saphenous nerve below the knee. Observation B was made four minutes after A. Note the summation of stimuli, in each case six stimuli being required before the reaction was evoked.

This time is spent in two ways ; first, and chiefly, in raising the c.e.s. of the neurones concerned, and secondly in true synaptic delay, which is probably quite brief. We find that central reflex times vary greatly according to the particular reflex ; *i.e.* to the number and properties of the neurones, and also according to the strength (Fig. 164), number and frequency of the stimuli given. The flexor response is a brisk one, with central delay of 3 to 6 msec. Contralateral reflexes usually occupy a longer time, but in all cases the stronger and more frequent the stimuli the shorter the latent period will be. We may suppose that this is because with stronger stimuli a given motoneurone receives a greater number of impulses from different synaptic knobs in a given time. When natural stimuli are given the afferent impulses are not volleys, but are dispersed, so that latent periods may be quite long.

(5) *Summation* of stimulation is a conspicuous feature of reflex actions. A single shock of a strength which would excite strong contraction of the tibialis anticus when applied to its motor nerve (the peroneal) is often found

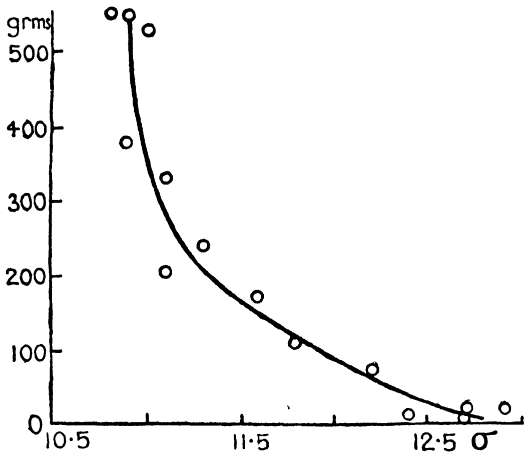

FIG. 164. Effect of Strength of Stimulus on Latent Period of Reflex Response Reflex excitation of tibialis anticus through popliteal nerve of same side. The strength of stimulus is indicated in the figure by the strength of the response. (CREED *et al* "Reflex Activity in the Spinal Cord" Oxford Univ. Press, 1932.)

to be without effect when applied to an afferent nerve such as the popliteal; but two or more such afferent stimuli, in quick succession, will, after a latent period, cause a reflex contraction (*temporal summation*). In some reflexes

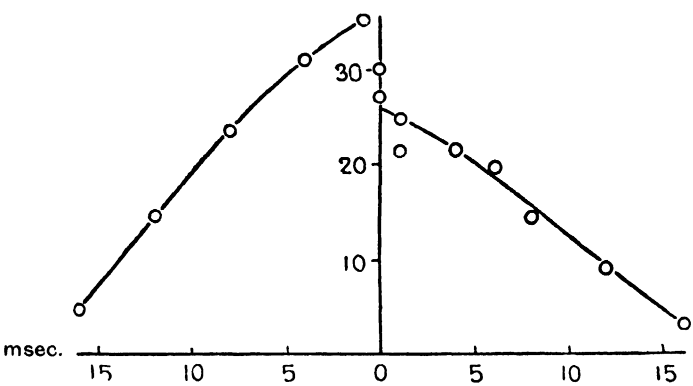

FIG 165. Reflex Contraction of *Tibialis Anticus* to Pairs of Threshold Stimuli separated by the Intervals shown on the Abcissæ. Ordinates, tension in g. The stimuli were given first to medial gastrocnemius nerve, second to lateral gastrocnemius nerve (on left of zero) and in reverse order (to right of zero). (CREED *et al.* "Reflex Activity of the Spinal Cord." Oxford Univ. Press, 1932.)

(*e.g.* scratch reflex) Sherrington found that as many as forty stimuli may be needed to release a reflex, and the latent period may then amount to seconds. Similarly, two stimuli, each of which gives only a small response, may summate to give a larger one.

Summation may be obtained when two stimuli are applied to different afferent nerves, and experiments on summation are usually made in this way (Fig. 165), because then there can be no question of the nerve fibres being in a refractory state following the first stimulus. If the two stimuli are simultaneous, this type of summation is sometimes called *spatial summation* or ' immediate induction.' Its occurrence illustrates the fact that different afferent paths may converge to a final common path. The facts of summation are represented diagrammatically in Fig. 166.

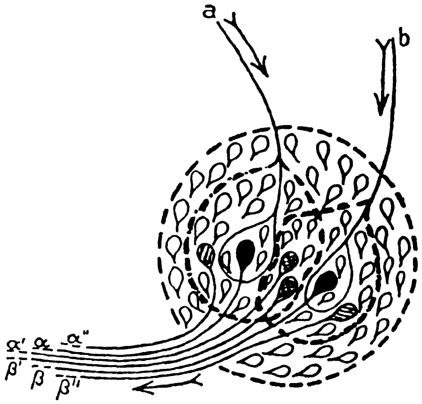

FIG. 166. Two Afferent Fibres are shown entering a Motoneurone Pool. The continuous line shows the field of threshold action (one neurone excited in each). The dotted lines inside the pool show the subliminal fields (three neurones in each). Each fibre alone excites only one neurone ; concurrently stimulated four neurones are excited, *i.e.* one from each threshold field and two from overlap of the subliminal fringes. (After CREED *et al.* " Reflex Activity in the Spinal Cord.")

It will be seen from this figure that when a fibre is excited, in addition to affecting certain neurones in the pool, an area of subliminal c.e.s., called the *subliminal fringe*, is created around these. When a second fibre commanding the same reflex is stimulated at the same time, it also creates a subliminal fringe, and if and where these adjacent fringes overlap, the result is that the c.e.s. there will rise to a sufficient level to cause excitation.

If the two (or more) stimuli, instead of being given simultaneously, are given in succession, summation (temporal summation, or ' successive induction ') can still occur if the interval between them does not exceed a certain amount, in the case of the flexor reflex about 15 msec. (Fig. 165). This may be interpreted as meaning that the c.e.s., which, as we have seen, takes about 4 msec. to be established, will all have declined after 15 msec. Naturally the greatest effects of two stimuli are seen when they are simultaneous, because then the c.e.s. due to each reaches its maximum at the same time.

Owing to the facilitating effect of the first volley, the central reflex time of the response to the second is shortened even to less than 0·5 msec.

It will be clear that the explanation of summation is to be found in the facilitation phenomena which we have already studied. The succeeding stimuli no doubt open up delaying by-paths through internuncial neurones until finally a sufficient number of impulses arrive in a given time at the motoneurones concerned.

Successive spinal induction is said to be positive when a previous stimulus favours a succeeding one, as in the examples we have mentioned. In some cases, however, where two reflexes are opposed, a reflex will favour the appearance of the opposing one. Thus a flexor reflex may be followed by an extensor movement, thus revealing a pre-existing state of subliminal extensor excitation. This is called *negative successive induction*, and such phenomena are probably responsible for rhythmic reflexes such as those of stepping.

(6) *After-discharge.* When the motor twitch of the tibialis anticus caused by stimulation of its motor nerve by a single shock is compared with the reflex contraction caused by a single powerful shock to the popliteal

nerve, it is seen that the reflex contraction lasts for about 0·1 sec. longer than the motor-nerve twitch. This is due to what is called 'after-discharge' and in extensor reflexes is very much more prolonged still.

It is also longer after repeated stimulation than it is after a single afferent

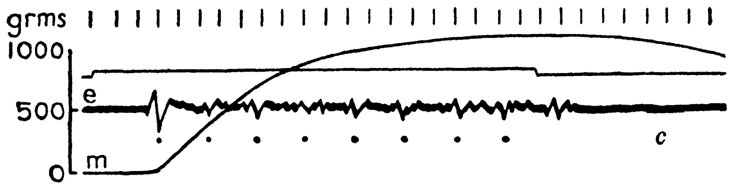

FIG. 167. Electrical and Mechanical Records of the Response of Tibialis Anticus to Repetitive Stimulation of Posterior Tibial Nerve. Frequency of stimuli marked by dots and large waves in electrogram; note the secondary waves. Time 10σ. (CREED *et al.* "Reflex Activity of the Spinal Cord." Oxford Univ. Press, 1932.)

shock (Fig. 167), and when electrical records are also made, it is seen that there are nerve impulses after stimulation of the afferent nerve has ceased.

The cause of after-discharge has already been discussed when dealing with the properties of central conduction. It is due to the fact that the impulses travelling to the motoneurones are delayed to varying extents.* Some arrive almost direct, others are delayed in internuncial by-paths and reach the motoneurones later (Fig. 168); if the refractory period of these has passed off these later impulses may cause them to fire off another impulse. Hence there are more efferent impulses than afferent stimuli (Fig. 167); with strong stimuli some of the later impulses also reach motoneurones not previously affected, and so the motor discharge is prolonged.

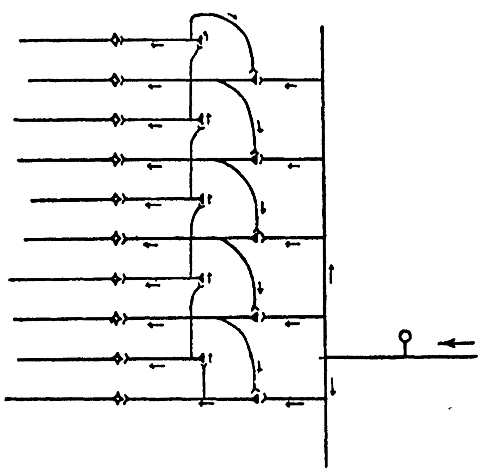

FIG. 168. Diagram to Illustrate Causation of After-discharge by Passage of Impulses along Delay and Re-exciting Circuits. Arrows show directions of impulses but not their time-relations; these latter depend on the number of synapses passed. (RANSON and HINSEY. *Amer. J. Physiol.*, 930, **94**, 471.

If an antidromic volley is fired back from the motor nerve, the after-discharge lapses for from 20 to 60 msec. and then reappears; this fact is in accordance with the explanation given above, the antidromic volley serving to discharge all the motoneurones simultaneously. After the quiet interval belated impulses again succeed in causing excitation.

(7) *Refractory Period.* The neurones, after having discharged an impulse pass through a refractory period, but this is not easily measured with accuracy. The best method is to apply an antidromic stimulus to the

* FORBES. *Physiol. Rev.*, 1922, **2**, 361.

motor nerve * (Fig. 169). This causes all the motoneurones to discharge, and it is found that for about 11 msec. afterwards afferent stimuli are ineffective. Hence the refractory phase is assessed at about 11 msec. Longer periods of irresponsiveness in reflexes occur, but are usually attributed to inhibition, *i.e.*, the neurones are not refractory, but are probably not receiving enough afferent impulses.

(8) *Fractionation.* When a given afferent nerve is stimulated with a maximal shock the reflex contraction of a muscle is only a fraction of what it is capable of producing when its motor nerve is stimulated directly. Thus a given tibialis anticus muscle could exert a tension of 2160 grammes when directly stimulated. When reflexly stimulated through the internal saphenous nerve the tension was only 800 grammes, when by the musculocutaneous branch of the peroneal, 1700 grammes ; by the external plantar 1240 grammes ; by the internal plantar 1330 grammes ; by the small sciatic 680 grammes, and so on. This phenomenon is called 'fractionation.' It means that each afferent nerve can only command a portion of the motoneurone pool that supplies the muscle.

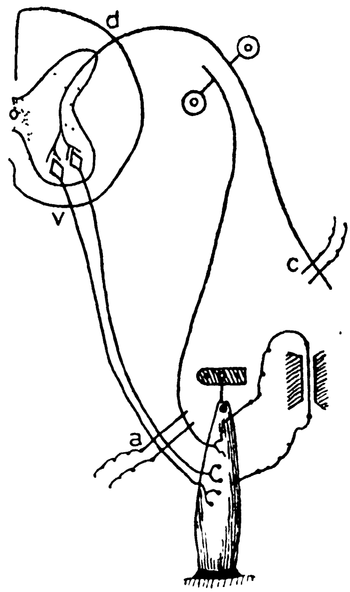

FIG. 169. Schema of Arrangement for Firing Antidromic Volley. v, d, ventral and dorsal roots; a, electrodes on motor nerve; c, electrodes on an afferent nerve Needle electrodes stuck into muscle lead to a string galvanometer for recording action potentials. (CREED *et al.*)

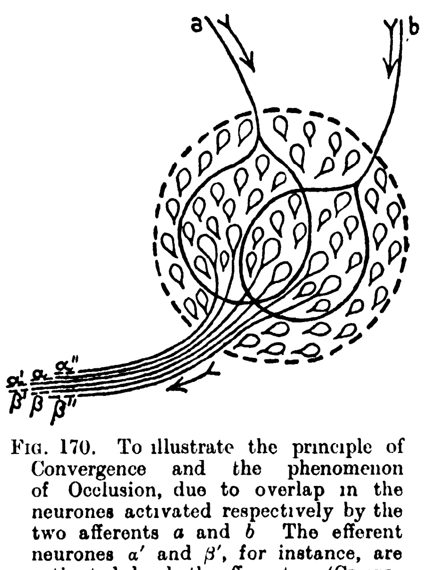

FIG. 170. To illustrate the principle of Convergence and the phenomenon of Occlusion, due to overlap in the neurones activated respectively by the two afferents *a* and *b* The efferent neurones α' and β', for instance, are activated by both afferents. (CREED, DENNY BROWN, ECCLES, LIDDELL & SHERRINGTON, *Reflex Action of the Spinal Cord* Oxford University Press, 1932)

It should be noted, however, that the *sum* of the tensions obtainable from *all* the afferents greatly exceeds the maximal tension obtainable on motor nerve stimulation. In the above example the tensions produced by separately stimulating ten different afferent nerves, added up to 8370 grammes, whereas the motor tetanus gave 2160 grammes. This is readily explicable as an example of the *Principle of Convergence,* the areas of influence of the various afferents overlapping considerably, as illustrated in Fig. 170.

(9) *Occlusion.* The phenomenon called occlusion is another consequence of the principle of convergence. When reflex contraction of a muscle is produced by the

* ECCLES. *Proc. R. S.*, 1931, 107B, 557.

simultaneous stimulation of two afferent nerves, the resulting tension is usually definitely less than the sum of the tensions set up by stimulation of the two nerves separately. Thus, when either of two plantar nerves was stimulated, 1570 grammes tension was produced in a tibialis anticus muscle. If both nerves were stimulated one might therefore expect a tension of 3140 grammes, but that actually developed was only 1810 grammes, *i.e.* 1330 grammes less. This phenomenon is again due to certain of the motor neurones being stimulated by both afferent nerves. Whereas one plantar nerve causes reflex stimulation of x motor neurones, and the other plantar nerve causes the reflex stimulation of y motor neurones, the two nerves together do not activate $x + y$ motor neurones because some of those included in x are also included in y, *i.e.* there is considerable overlap, or 'occlusion.'

Occlusion is greatest in closely allied afferent nerves, because they are more likely to control adjacent parts of the motoneurone pool; it is also greater with more powerful and numerous stimuli, because these extend the area controlled by each afferent and so increase the overlap.

We may now consider some other characteristics which are seen to greater advantage in reflexes other than the flexor.

(10) *Inhibition.* This can be elegantly demonstrated on the crossed extensor reflex. If the central end of the contralateral sciatic nerve is stimulated by rapid shocks the quadriceps extensor enters into sustained contraction. If, during this tonic state, the central end of an ipsilateral afferent nerve, *e.g.* popliteal, be strongly stimulated, the extensor contraction is instantly abolished. In a similar way the flexor reflex which is excited by

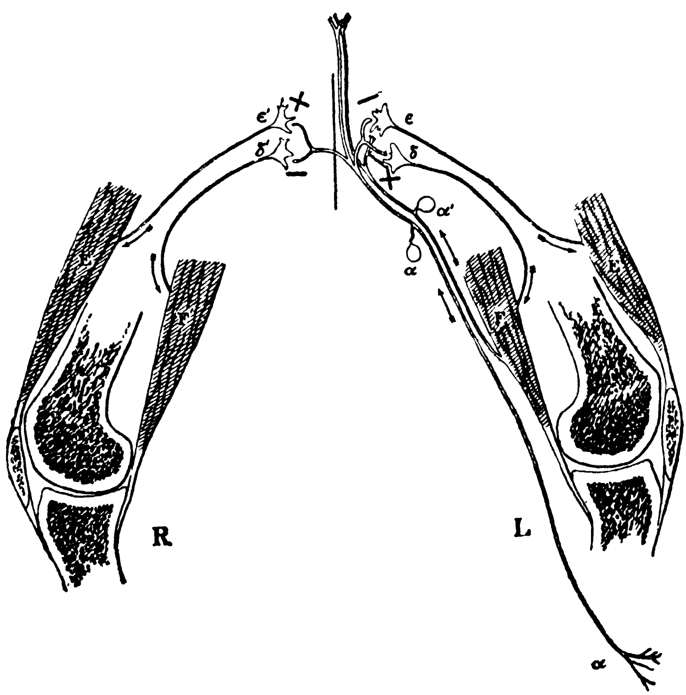

FIG. 171. Diagram indicating Connections and Actions of two Afferent Spinal Root Cells α and α' in regard to their reflex influence on the extensor and flexor muscles of the two knees. The sign + indicates an excitatory effect, the sign — an inhibitory effect. (SHERRINGTON.)

an ipsilateral afferent can be inhibited by stimulating a contralateral afferent. It will be evident that this phenomena of central inhibition, *e.g.* of an extension by a flexor reflex, must be closely related to that of reciprocal innervation, and results in a smooth and economical functioning of the muscles; the afferent nerve which causes reflex flexion, at the same time inhibits extension, and *vice versâ*.

The anatomical basis of the various events involved in the carrying out of such a reflex as that just studied is shown in Fig. 171. In this diagram the nerve fibre α represents the nociceptive nerve from the skin of the foot. This passes by a dorsal root into the spinal cord, where it gives off a number of collaterals. Those collaterals, which affect the motoneurones to the two hind limbs, can be divided into two sets, one of which produces excitation in some neurones, whilst the other produces inhibition of the motoneurones to the antagonistic muscles. The single afferent nerve fibre is, therefore, with regard to one set of its central terminal branches, specifically excitor and, in regard to another set, specifically inhibitor. In the case in point the central terminal branches of the nerve α are excitor for the flexor and inhibitor for the extensor muscles of the same side, and the reverse for the muscles of the opposite side.

The diagram shows also that the contraction of the flexor muscle itself initiates a secondary reflex process from the muscle up the nerve fibre α' and back again to the muscle by the efferent neurone. This muscular afferent nerve also has central terminations of two signs—excitor to itself and inhibitor to the antagonistic muscles.

It is important to note that the efferent path innervating, say, the flexor muscles is common to many reflexes. It is used, for instance, by mutually exclusive reflexes such as the 'scratch reflex' and the 'flexor reflex.' Evidently the mutual inhibition of opposed reflexes occurs, not in the 'final common path'—*i.e.* in the motor neurones, which must always remain open—but further back in the arc, probably near its afferent side.

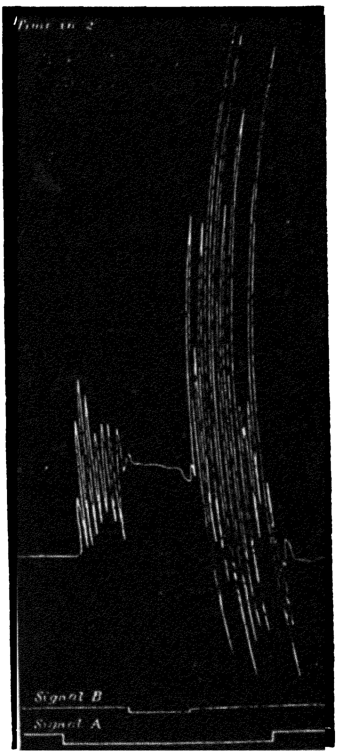

FIG. 172. Scratch Reflex temporarily inhibited by Application of a Nociceptive Stimulus to Foot.

Signal A, stimulation of scratch area. Signal B, stimulation of paw by strong induction shock.

The biological value of inhibition is clear. For proper co-ordination there must be no clashing between opposing activities of different parts. One stimulus may *reinforce* another if the reactions ensuing on the two stimuli are *allied*—*i.e.* tend to co-operate one with another. But if one part is engaged in any action, this action must be the policy of the body as a whole. Yet the surface of the body is being continually played upon by ever-changing stimuli, tending to excite first one reflex and then another, and the activities so excited would produce confusion in the conduct of the animal, if there were not some means by which at any one time only one reaction should be carried out. The imperative stimulus should dominate the actions

of the body as a whole. There may be a struggle of different stimuli, but one must finally be *prepotent* and annul altogether the influence of the others. The study of the spinal reflex shows that this end is obtained by the process of inhibition. Every successful reflex inhibits all other reflexes which are not co-operative with it. We may, for instance, stimulate the area of skin which gives rise to the scratch reflex, and at the same time apply a painful stimulus to the foot. The result is not a movement compounded of the two reflexes, but the scratch reflex immediately comes to an end, and the flexor reflex occurs. When this in its turn stops, the scratch reflex may be once more resumed (Fig. 172).

Central Inhibitory State. Inhibition is believed to be produced as a result of the establishment in the centres of a condition called the *central inhibitory state* (c.i.s.), which is antagonistic to the c.e.s. As we have already seen, all

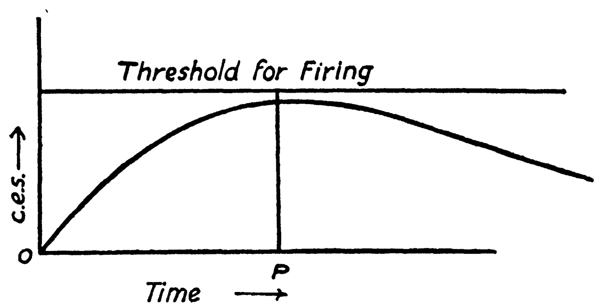

FIG. 173. Probable relation between time and intensity of c.e.s. The moment P, when *c.e.s.* is at a maximum, is about 6σ after the arrival of the impulse at 0.

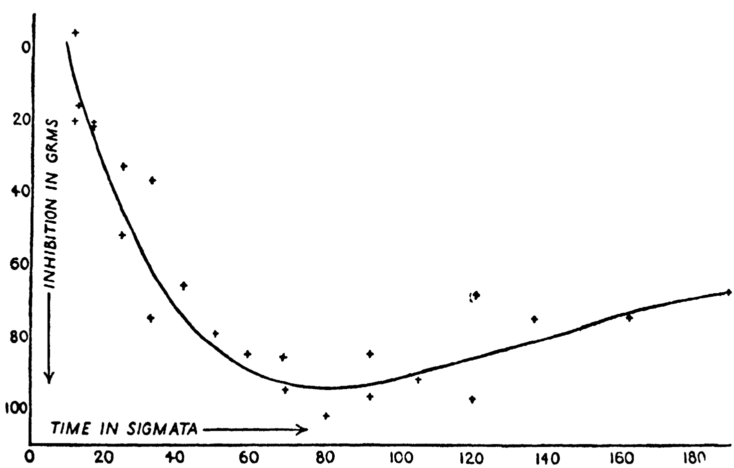

FIG. 174. Tensions of Reflex Twitches (Ordinates) Plotted against Intervals by which an Inhibitory Volley precedes the Excitatory (abscissæ). (CREED *et al.* "Reflex Activity of the Spinal Cord.")

the phenomena of inhibition may be due to nothing more than a lack of facilitation. If an inhibitory volley *precedes* the excitatory one, the extent of the resulting inhibition depends on the time interval between them. It is maximal (for the inhibition of the flexor reflex) at about 80 msec. (Figs. 173 and 174).

The c.i.s. produced by an afferent stimulus thus rises more slowly than c.e.s. (which is at a maximum in about 6 msec.). It also lasts longer, and then slowly subsides.

The c.i.s. can be summated, either temporally or spatially. Thus two inhibitory stimuli produce more inhibition than one, if suitably spaced. An antidromic volley cannot, however, discharge c.i.s. as it can c.e.s. Excitation and inhibition can be quantitatively balanced one against the other and the stronger can ultimately overcome the other (Fig. 175).

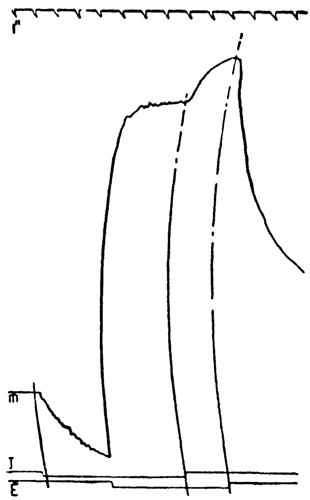

FIG. 175 Algebraic Summation of Excitatory and Inhibitory Reflexes in Vastocrureus of Decerebrate Cat. The muscle begins in extensor tone. At *I*, stimulate ipsilateral peroneal. At *E*, stimulation of contralateral popliteal. Note increase of contraction, due to release from inhibitory influence, when *I* ceases (CREED *et al.* "Reflex Activity of the Spinal Cord.")

The exact way in which inhibition is produced has been the subject of many speculations. Many kinds of inhibitory phenomena are met with in the body, and the explanation of one may not be applicable to another. Recent evidence seems to point to the probability that the neurones of the central nervous system are normally exposed to constant subliminal bombardment with impulses and that excitation occurs when new impulses arrive in sufficient numbers and at appropriate instants. Inhibition then comes about if this background of facilitation is withdrawn, because, as we have seen, it needs more than one impulse to excite a motoneurone to the discharge point.

An explanation of reciprocal innervation has been advanced by Gasser on the following lines (Fig. 176). Suppose that two synapses must be excited within 0·5 msec. in order to excite the neurones. When fibre I is repeatedly excited *b* and *c* and hence F, a flexor motoneurone, are stimulated.

Now, suppose that while this is happening, fibre II is stimulated. It has three synaptic knobs on *b* (and two on *a*), and can excite *b* at its own rhythm, which is different from that of I. Neurone *b* now comes under the control of II, and discharges at a different rate from *c*, so that F does not receive successive impulses from *b* and *c*, within 0 5 msec. and therefore ceases to respond. Neurones *a* and *b*, however, act in unison and so excite E, causing contraction of the extensor.

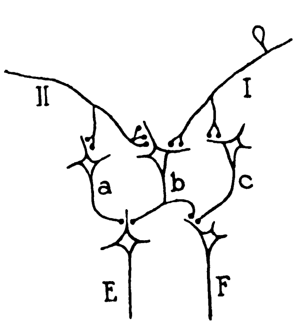

FIG. 176. To illustrate Theory of Central Inhibition and Reciprocal Innervation. (GASSER. Harvey Lecture, 1937.)

(11) '*Rebound.*' Inhibition is often followed by a condition of increased excitability, as a sort of swing-back of the state of excitation. Thus, suppose the scratch-reflex is excited and then, while the excitation is still continued, the reaction is inhibited by excitation of the flexor reflex. As soon as the flexor reflex has passed off, the scratch reflex returns with an intensity greater than before (Fig. 172, p. 271). It may also be seen sometimes as an increased after-discharge following the cessation of an excitatory stimulus. In the knee-jerk a succession of inhibitions and rebounds may result in a clonic contraction.

(12) *Negative Successive Induction.* When a reflex subsides, it often tends to be followed by an opposing movement, a phenomenon called

negative successive induction. This explains the tendency of the spinal cord to an alternation of response; every act tending as a result of negative spinal induction to induce the antagonistic act. Thus if a spinal dog be held up vertically, so that the hind limbs hang freely, these latter execute a series of alternate movements of flexion and extension—the '*mark-time*' *reflex.* The starting point of these is the stretching of the anterior thigh muscles. Once started they continue of themselves, each act exciting the alternating antagonistic act (Fig. 177).

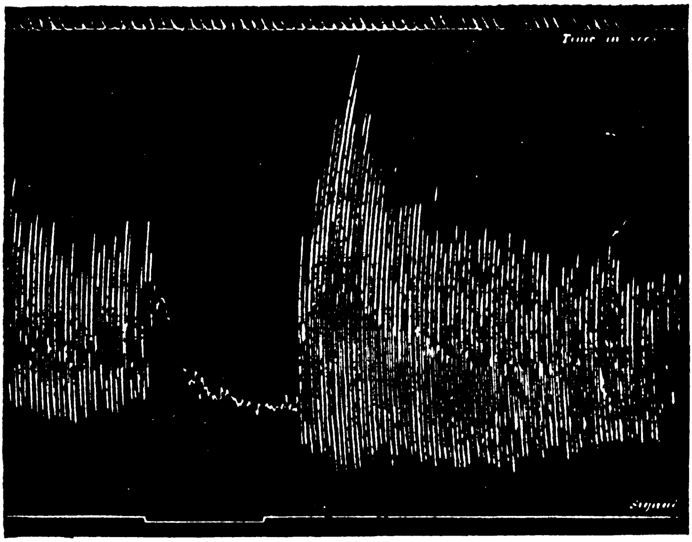

FIG. 177. 'Mark-time' Reflex in Spinal Dog, inhibited by slight Stimulation of the Tail (duration of stimulation shown by signal). Note the augmentation of the mark-time reflex following the inhibition (*rebound*). (SHERRINGTON.)

The movements of biting and mastication are probably largely effected by a series of rebounds aided by negative successive induction, originated by the mechanical stimulus of food in the mouth.

(13) *Recruitment.* Many reflexes, especially the extensor ones, show a much slower development than does the flexor response or that obtained by stimulation of the motor nerve; this is due to the fact that the motor units are not thrown into action all at once, but are brought into play in regularly increasing numbers up to the maximum. The decline of these reflexes is also gradual. This phenomenon is called *recruitment,* and is responsible for the steady rise and fall of contraction in certain natural movements, such as those of the respiratory muscles.

When, as in many flexor reflexes, all the motor units employed are thrown into use at the commencement of the response, the onset is sudden, and is of the type called by Sherrington '*d'emblée*' reflexes. Fig. 178 shows the striking difference between flexor and extensor reflexes.

It will be seen that the flexor response shows less delay, needs a weaker stimulus, has a more abrupt rise and fall, has little after-discharge, and shows the rhythm of the afferent stimuli. The slow falling-off of the extensor response is clearly due to prolonged after-discharge. Its slow rise, or recruitment, is probably explicable as due to a larger number of internuncial neurones in the reflex arc. Hence the delay in commencement is longer than

for the more direct flexor reflex, because the c.e.s. takes longer to work up to an excitatory level; it also more slowly reaches a maximum as the later impulses trickle into the motoneurone pool. Also, owing to the many

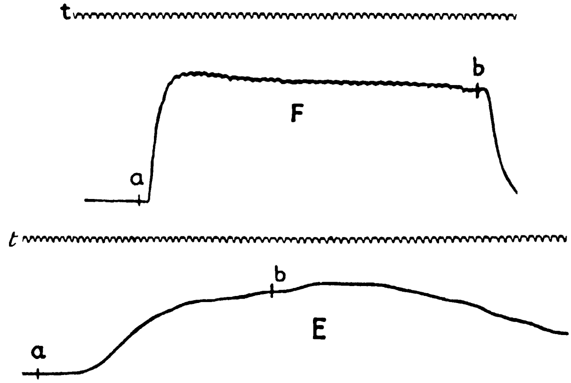

FIG. 178 Flexor reflex (F) from Semitendinosus on Stimulation of Ipsilateral Popliteal Nerve Coil at 16 8 cm Extensor reflex (E) from vastocrureus on stimulation of contralateral popliteal nerve Coil at 15 cm Stimulation in both cases between *a* and *b* at 38 p. sec. (From LIDDELL and SHERRINGTON. *Proc R S*, 1923, **95B**, 299)

different delay paths the rhythm of the afferent stimulus is damped out, so that a smooth contraction results even when the rate of afferent stimulation is relatively slow.

Inhibition, as well as excitation, shows the phenomenon of recruitment, if the inhibitory stimulus is weak, *i e.* the motoneurones cease working in succession, not all at once (Fig. 179); it also shows after-discharge.

There is an alternative explanation of recruitment. The extensors in the decerebrate preparation are normally in a state of tone, due to stretch-reflexes. When an extensor contraction begins, the stretch is relieved, so that the rise of tension is slowed down: also, afferent impulses having an inhibitory effect pass up from the muscle, and these have the same effect of acting as a brake on the rise of tension. The converse happens

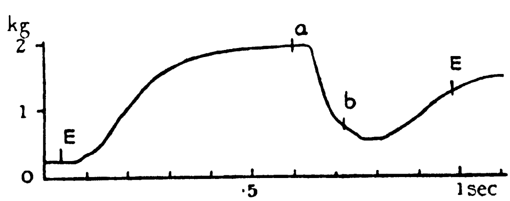

FIG. 179. Recruitment and 'after-discharge' of Inhibition. *Rectus femoris.* E to E, stimulation of contralateral afferent causing crossed extensor reflex. *a* to *b*, stimulation of ipsilateral afferent causing inhibition of slow onset and outlasting the stimulation. (LIDDELL and SHERRINGTON, *Proc. Roy. Soc.*, 1925, **97B**, 488)

when relaxation occurs. The evidence for this view is that if the afferents from the extensor muscles are divided, the characteristic recruitment and after-discharge are much lessened, and hence they must be partly proprioceptive in origin.*

The extensor muscles therefore tend to give smooth contractions and steady relaxations.

(14) *Fatigue.* If a given reflex be repeatedly elicited, the reaction becomes feebler and finally disappears altogether, long before any signs of

* SHERRINGTON. *Q. J. Exp. Physiol.*, 1909, **2**, 109.

fatigue in the motor apparatus can be detected on stimulation of the motor nerve. Since we know that it is practically impossible to fatigue nerve fibres, we must conclude that the seat of fatigue is in the grey matter of the spinal cord itself.

We have reason to believe that the propagation of impulses through the central nervous system involves expenditure of energy, and that the seat of greatest expenditure may be located at the synapses. It follows that the result of any particular sensory stimulation will not be absolutely invariable, but that the spread of the impulses in the nervous system, and the degree of block presented by the various synapses, will depend on their condition at the time.

This condition may be altered in various ways. Repeated excitation causes in the synapses, just as in the sensory and motor nerve-endings, a condition of fatigue. Stimulation confined to a single point in the " scratch area " of the spinal dog excites a scratch reflex which rapidly dies away. On shifting the exciting electrodes a little to one side, the reflex act begins again, often with greater force than at first ; and a very prolonged reaction can be induced by gradually moving the electrodes along the surface of the skin. Some reflex arcs rapidly show signs of fatigue, and the minute change in locus of stimulus, which is required to re-induce a practically identical action, shows that the seat of fatigue must lie chiefly on the afferent side of the arc, perhaps in the quickly adapting sensory nerve-endings, as well as in the first synapses through which the impulse has to pass. This easy incidence of fatigue tends to cut short some reflexes and to render it easier for other reactions to take their place.

(15) *' Block ' or Resistance.* As we have seen, there is an absolute block to the passage of an impulse backwards through a synapse. The phenomena of fatigue show that there may be resistance even to the passage of an impulse in the normal direction. When we study the structure of the central nervous system, we find that although there are certain shortest possible paths for every impulse, yet so extensive is the branching of the entering nerve fibres and so complex are the neurone systems with which they come into connection, that if all resistance were removed, an impulse entering along any given fibre could spread to practically every neurone in the spinal cord and brain. Such a result is indeed observed in animals poisoned by strychnine. In these, the slightest stimulus applied to any part of the skin excites strong tonic spasms in the musculature of the whole body. Every sensory nerve fibre, that is to say, can discharge into every motor neurone of the cord. That this result does not ensue on localized stimulation in a normal animal is because of the varying resistance to the passage of an impulse into the several neurones with which the entrant fibre comes into anatomical relation. A small stimulus will discharge only along the few neurones where the resistance is lowest. Increase of the stimulus, either by adding to its strength or by summation of weak stimuli, will enable the impulse to irradiate and therefore will elicit a more widespread response. Only when the ' blocks ' are entirely removed by the administration of strychnine, or when the stimuli are abnormally powerful and long continued, will the impulse spread to all regions of the central nervous system, so that the response becomes general and inco-ordinate.

STRETCH REFLEXES

Normal muscles are in a state of slight constant tension, or *tone,* and in the decerebrate animal this is greatly exaggerated in the extensor muscles.

Section of either the motor or the afferent nerves of the muscle destroys this tone and the muscle becomes limp. When the tendon of such a denervated muscle is pulled upon, the muscle is merely passively stretched. It is quite different if a pull is given to the tendon of an innervated extensor muscle, such as the quadriceps, of a decerebrate preparation. The muscle in such a case returns pull for pull, actively contracting against and in proportion to the stretching forces. This response is a *stretch reflex.* Such an experiment is illustrated by Fig. 180. The quadriceps of a decerebrate cat was attached by the patellar tendon to an isometric lever and was subjected to stretching by pulling the animal in the opposite direction to the lever by lowering the animal table by a few millimetres.

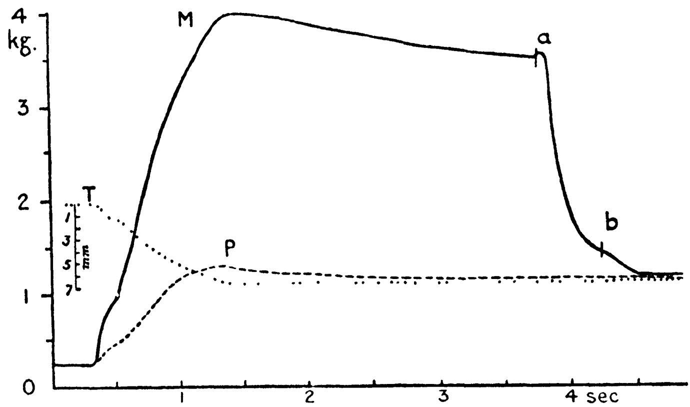

FIG. 180. Stretch Reflex of *Quadriceps*, decerebrate Cat. Extent of stretch shown by curve T ; M, tension of muscle ; P, passive tension by repeating stretch after denervation of muscle. *a*, *b*, inhibition by stimulation of ipsilateral popliteal nerve. (LIDDELL and SHERRINGTON, *Proc. Roy. Soc.*, 1924, **96B**, 212.)

A tension of nearly 4 kg. resulted, but after denervation the passive lengthening of the muscle by the same stretch gave only a little over 1 kg. tension. The reflex and proprioceptive character of the pull is demonstrated by the fact that it disappears completely if the afferent fibres from the muscle are divided by section of the appropriate dorsal nerve roots.

The stretch reflexes are shown especially well in the extensors of decerebrate preparations, but sustained stretch reactions are not seen in flexors. These proprioceptive reflexes originate in the stimulation of the stretch receptors in the muscle and tendon. As we have already seen (Chapter XIV) the stretch receptors start off trains of impulses when the muscle is stretched, and as the stretch increases the impulses follow each other more frequently and along more and more fibres. Muscle stretch receptors do not adapt rapidly and so keep up trains of impulses along the afferent fibres from the muscle as long as the stretch is maintained, and if this is increased, more and more motoneurones are recruited. The contraction is therefore as sustained and as strong as the stretching force.

The pain receptors, by way of contrast, adapt quickly, and so are more suited to evoke the brisk but more evanescent flexor responses.

Stretch reflexes have short latent periods (6 to 20 msec.) and show little after-discharge. They can at once be inhibited, as Fig. 180 shows, by exciting an opponent reflex. The rate of discharge of the motoneurones is slow, *e.g.* about 8 p. sec. It is probably on this account that fatigue does not occur.

Types of Muscle. Most of the pure flexor muscles are made up of pale fibres, the rapid contraction of which is suited to their function. Extensor muscles, on the other hand, are capable both of slow, sustained contractions, as in stretch reflexes, and of quick ones, and accordingly consist of two parts, a quick acting part, of pale muscle, and a slow one of red muscle.* Thus, soleus and gastrocnemius extend the ankle, the former slowly, the latter quickly; medial short head of triceps extends the elbow slowly and the lateral short head quickly, and so on. Physiologically they are distinct muscles, though often associated anatomically as separate 'heads.' Usually the slower muscle is the more deeply placed.

The stretch-reflexes have great importance in connection with the maintenance of posture, of which they form the background. They are seen in those muscles that normally counteract the effect of gravity, and their tonic nature enables an animal to maintain its normal posture without fatigue. When an animal stands erect, gravity tends to cause flexion at hip, knee and ankle, but any tendency of the limbs to give way is instantly corrected by the stretch reflexes, which convert the limbs into rigid pillars.

TENDON-REFLEXES

The knee-jerk, and other examples of 'tendon reflexes' already referred to in Chapter XIII are special cases of the stretch reflex. In them the tug on the muscle is very brief and the response is brisk. They originate from the stretch of the muscle and not from the tendon, since they can still be elicited after cocainizing the tendon. The latent period is very short (6 to 8 msec.) because a synchronous volley is sent up from the muscle, and because the arc is short.

The electric potential changes during a tendon jerk show a burst of impulses immediately preceding the contraction, followed by a sudden cessation—the 'silent interval' at its height, and then a further train during and after the peak. The silent period has been shown to be partly due to the relief of tension on the stretch receptors when contraction has occurred, and partly to reflex inhibition. The tendon jerk can be abolished by inhibitory excitation, *e.g.* the knee jerk by stimulation of an ipsilateral afferent.

The reason why tendon jerks and stretch reflexes are more easily shown in the decerebrate preparation is probably that there is a constant state of extensor excitation facilitating the responses. In man the knee jerk is facilitated, or 'reinforced,' by clenching the fists.

LENGTHENING AND SHORTENING REACTIONS

If one attempts forcibly to flex one of the rigidly extended limbs of a decerebrate preparation, the resistance due to the stretch reflex is at first encountered, but if the pressure is continued, the limb quite suddenly folds up—'*clasp-knife reaction.*' This sudden yielding in the face of threatened rupture is a protective response and is called the *lengthening reaction.* It is due to the fact that at a certain degree of tension inhibitor afferents gain the ascendancy. Accompanying this inhibition is a tonic increase of extension in the opposite limb, which is called Philippson's reflex (Fig. 181). It is akin to the crossed extensor reflex.

Like the crossed extensor reflex it is converted from a tonic to a phasic response if the extended muscle is first deafferented (Fig. 181.D). This shows that in some manner the extensor reflex is maintained in a tonic state as a result of afferent impulses from the extended muscle—possibly from its own stretch receptors.

The converse of the lengthening reaction is the *shortening reaction* seen if, after passive flexion, the limb is passively re-extended, when it 'stays put' in extensor tonus

* DENNY-BROWN. *Proc. Roy. Soc.*, 1929, **104B**, 371.

as at the start. By these two reactions a certain plasticity is imparted to the exaggerated postural tonus of decerebrate rigidity.

There is evidence that when a muscle is stretched two opposing reflexes compete. The tendon receptors originate impulses which have an inhibitory effect on contralateral

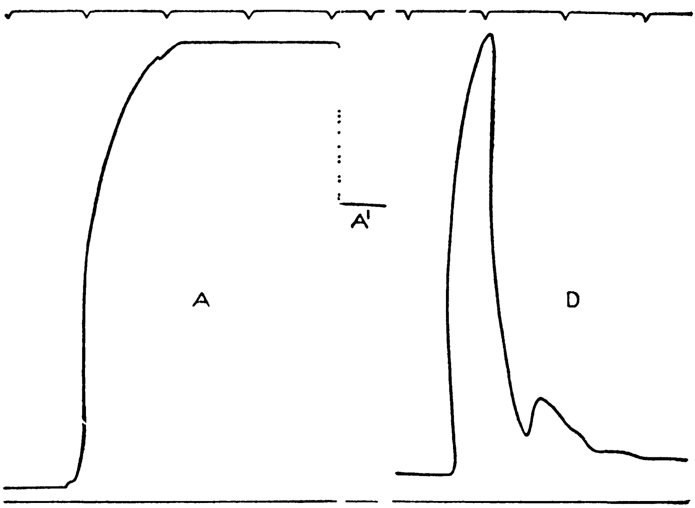

FIG 181. A Philippson's Reflex, a Contraction of the Extensors of a Decerebrate Preparation when the opposite Limb is forcibly bent at the Knee. A[1] the same 1 minute later D, the response 48 days after section of afferent roots of the muscle. Time = secs

extension, while the annulo-spiral spindle endings of muscle have an excitatory one, provoking the Philippson reflex.*

Ipsilateral Extensor Reflexes

Although the commonest type of reflex elicited by the artificial stimulation of afferent nerves consists in ipsilateral flexion, with contralateral extension, there are, nevertheless, various reflexes of ipsilateral extension. Some of these play an important part in the natural co-ordination of reflexes due to the normal stimuli to receptors. The stretch reflex, and the tendon-reflexes are examples of this : the extensor thrust and the positive supporting reactions are others.

An ipsilateral extensor reflex is also sometimes obtained on artificial stimulation of an afferent nerve, particularly of one placed distally on the limb. In these circumstances, the contraction of the extensor muscle precedes the normal inhibitory response accompanying the flexor reflex. It is believed that this extensor contraction competes, usually unsuccessfully, with the more powerful inhibitory one, so that it is only sometimes evident, and then often only for a time.

It would seem that each afferent nerve can excite two opposing reflexes, of which one is normally the stronger ; the other is called a '*concealed reflex*.' The motoneurones are always subjected to continual processes of excitation and inhibition, and their activity at any moment represents the algebraical sum of these opposed influences.

Reversal of Effect. Owing to the existence of concealed reflexes, it may happen that stimulation of an afferent gives first one reflex and then the reverse one. This may happen especially if the strength of stimulation is altered, or if the stimulation is prolonged, or the conditions changed, as by drugs. Terminal rebound is often such an instance, and possibly the rhythmic reflexes, such as stepping, the scratch reflex, etc., are others.

It was inferred by Graham Brown † that the spinal reflex centre for a limb consists of two ' half-centres,' one flexor and one extensor, and each afferent fibre reaching the centre divides into branches, some of which go to each half-centre.

* Morson and Phillips. *J. Physiol.*, 1937, **88**, 199.

† Graham Brown. *Q. J. Exp. Physiol.*, 1911, **4**, 331 ; 1912, **5**, 237.

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CHAPTER XVIII

THE LABYRINTH, AND POSTURAL REFLEXES

ALTHOUGH quite complex reflexes can be carried out through the mediation of the spinal cord, most of these reflexes are, in the intact animal, considerably modified owing to the action of the higher parts of the central nervous system. This process of modification consists of augmentation of some, and suppression of other reflexes, and a general grouping together of reflexes so that a co-ordinated response of greater complexity results. As an example of this kind of higher function we shall now consider the reflex aspects of posture maintenance. Although this involves the final employment of spinal arcs, the proper association of the various factors which are concerned is carried out largely by centres in the brain stem.

The various movements of the normal body are effected against a background of muscular tonus, which maintains the various postures. This state of tone is suitably modified when any movement takes place. Many of the movements of normal animals are effected reflexly, and their study provides a good example of the manner in which elementary reflexes, carried out by lower centres, are modified and integrated by the intervention of higher centres of activity.

The postural tonus of the musculature is also effected reflexly, being built up from afferent impulses from two chief sources, viz. the *labyrinths*, and the *muscle proprioceptors*, and from various subsidiary sources.

THE LABYRINTH

The primitive auditory sac arises as a simple involution of the surface. In the course of development the anterior part is modified to form the canal of the cochlea, which is set apart entirely for hearing. From the posterior

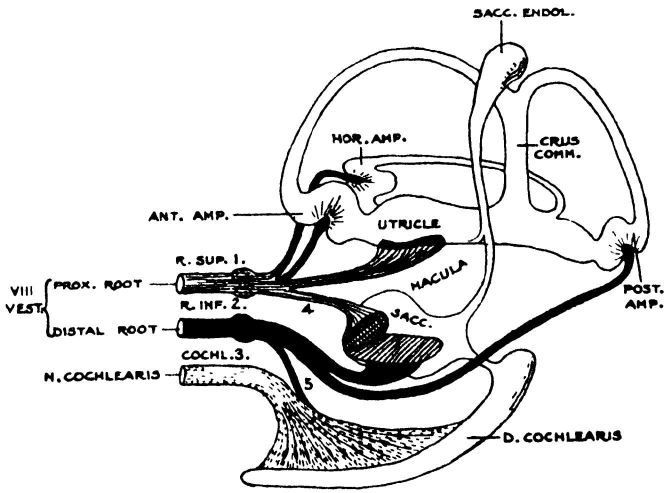

FIG. 182. Scheme of the Innervation of the Membranous Labyrinth. (H. M. de Burlet, *Anat. Anzeiger*, 1924.)

part there are formed two sacs—the saccule and utricle—and the three semicircular canals. The saccule and the utricle, which receive each a large branch of the vestibular division of the VIIIth nerve, represent the *otolith organ,* which is found in almost all classes of animals.

In mammals, that part of the labyrinth which represents the primitive otolith organs consists of a bony casing containing perilymph, in which is contained the membranous labyrinth, filled with endolymph. The osseous labyrinth consists of a cavity, the vestibule, into which open behind the three bony semicircular canals. In the vestibule are contained two little membranous sacs, the utricle and saccule, the cavities of which are connected by means of the *ductus endolymphaticus* (Fig. 182).

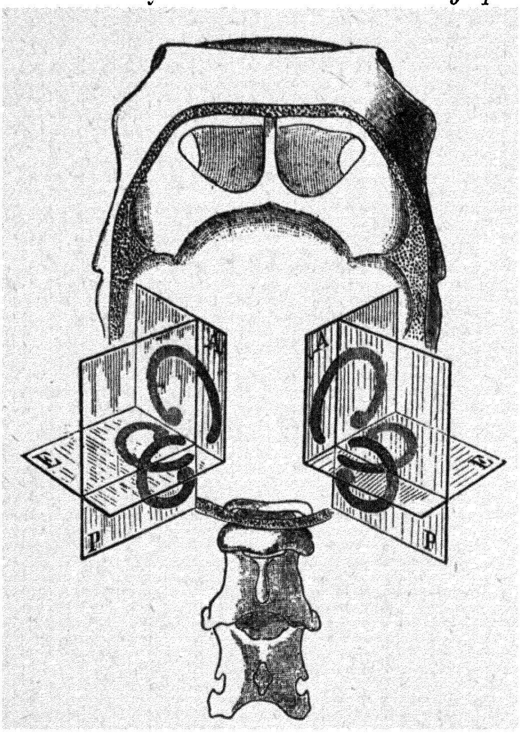

FIG. 183. The situations of the Semicircular Canals in the Skull of the Pigeon. (EWALD.)

Into the utricle, by five openings, open the three semicircular canals. These semicircular canals are arranged in three planes, each of which is at right angles to the other two, so that the three planes of space are represented. We may distinguish on each side an external or horizontal canal, an anterior vertical canal, and a posterior vertical canal. The two external canals on the two sides lie always exactly in the same plane, which in the normal position of the head slopes backwards and downwards at 30° to the horizontal. Each posterior vertical canal lies in a plane which is parallel to that of the anterior vertical canal of the opposite side. We thus see that these semicircular canals form together three planes, one nearly horizontal and two vertical, the two latter being at right angles to one another (Fig. 183). The membranous canal lies within the osseous canal, a considerable space intervening between the two canals. At one end each of the osseous canals is dilated and the membranous canal undergoes a corresponding dilatation so as to fill up the whole bony canal. In this dilatation, which is known as the *ampulla,* we find the ending of a branch of the vestibular nerve in a special sensory epithelium forming the *crista acustica* (Fig. 184). The crista is composed of hair cells with sustentacular cells between them. The fibres of the vestibular nerve end in arborisations among the hair cells, the hairs of which project into a dome-shaped mucilaginous body, the *cupola,* which lies in the endolymph filling the ampulla (Fig. 184).

In the utricle and saccule we also find in each an oval patch of modified epithelum. These are special receptor organs, known as the *maculæ acusticæ,* the structure of which is very similar to that of the crista in the ampullæ.

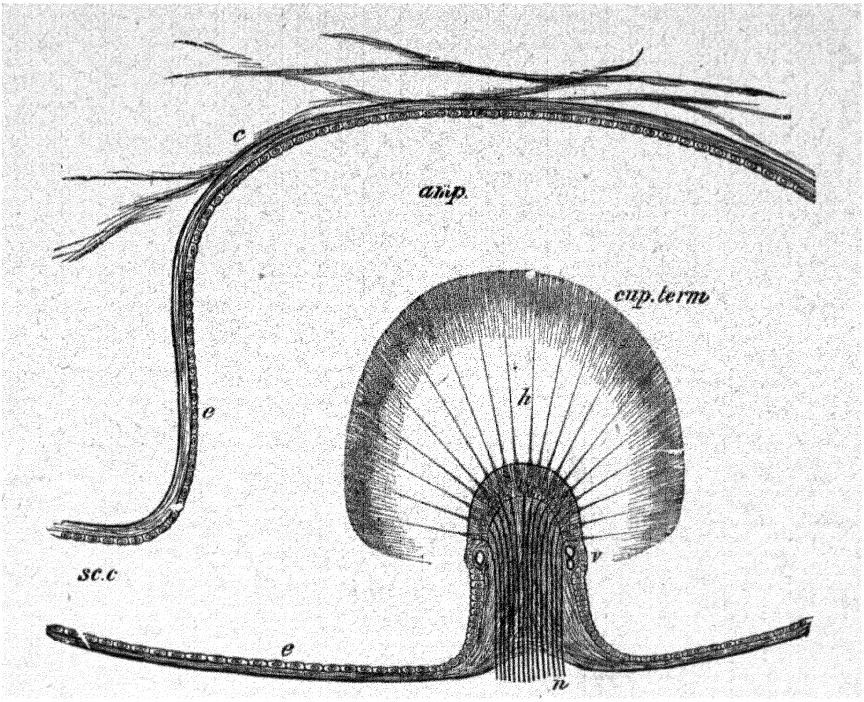

FIG. 184. End Organ (*Crista Acustica*) of Vestibular Nerve in Ampulla of Semicircular Canal. Cup. term., the *cupola*.

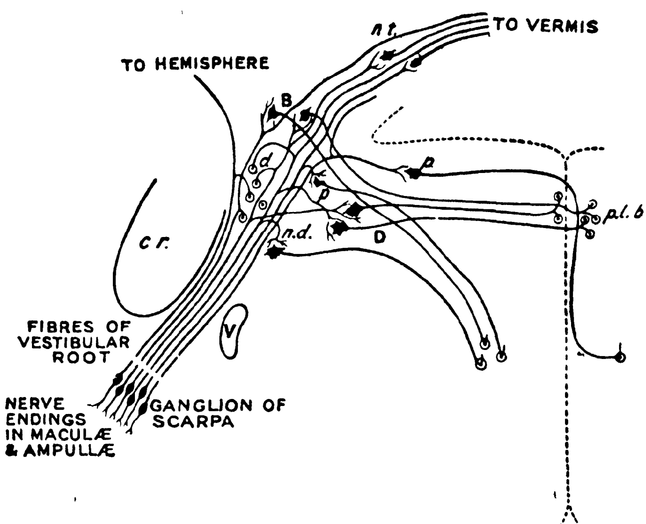

FIG. 185. Course and Connections of the Fibres forming the Vestibular Root of the Auditory Nerve. (SCHAFER.)

c.r., restiform body; V, descending root of fifth nerve; *p*, cells of principal nucleus of vestibular root, *d*, fibres of descending vestibular root, *n.d.*, a cell of the descending vestibular nucleus; D, cells of nucleus of Deiters; B, cells of nucleus of Bechterew: *n.t.*, cells of nucleus tecti (fastigii) of the cerebellum; *p.l.b.*, fibres of posterior longitudinal bundle. No attempt has been made in this diagram to represent the actual positions of the several nuclei. Thus a large part of Deiters' nucleus lies dorsal to and in the immediate vicinity of the restiform body.

In the mucilage covering the hairs of the macula, however, are found numerous small calcareous concretions, the *otoliths*. The macula of the utricle is in a horizontal plane, that of the saccule vertical.

NERVE PATHS CONCERNED. The vestibular part of the VIIIth cranial nerve has its cells of origin in Scarpa's ganglion. It enters the pons above the cochlear division and passes between the inferior cerebellar peduncle and the descending spinal root of the Vth cranial nerve. Its fibres end by arborizing round one of four different collection of nerve cells of which the lateral is called 'Deiters' Nucleus.' Fibres originating in these nerve cells form four pathways which convey impulses of importance in movements of the eyes, neck, and trunk ; in addition, large numbers of them go to the cerebellum (Fig. 185).

These paths are (i.) the *posterior longitudinal bundle*, to the oculomotor nuclei. These fibres, concerned with the co-ordinated movements of the eye muscles, run principally from the superior vestibular nucleus ('Bechterew's N.') and from Deiters' Nucleus ; (ii.) the *vestibulo-spinal tract*, descends to spinal motoneurones ; (iii.) the *vestibulo-cerebellar tract.*

Fibres conveying impulses to the cerebellum run principally from Deiter's Nucleus, but some go direct ; all of them enter the inferior cerebellar peduncle, relay in the n. fastigii, and are distributed to the cortex chiefly of the flocculonodular lobe of the cerebellum. From here impulses pass to the red nucleus, some back to the vestibular nuclei, and to various other destinations.

(iv.) The vestibulo-reticular tract to the bulbar nuclei.

THE FUNCTIONS OF THE LABYRINTH

The functions of the labyrinth may be divided into those of the vestibule, and those of the semicircular canals. They can be studied by the methods of extirpation, and stimulation. Both procedures result in disturbances of posture or of voluntary movements.

Unilateral labyrinthectomy in the rabbit leads to skew deviation of the eyes, nystagmus, rotation and lateral flexion of the head and spiral rotation of the neck and trunk towards the side of the lesion, diminution of tone of the limbs of the same side and increase in those of the opposite side, and frequently rolling movements, in which the animal turns over and over. In cats and dogs the deviation of eyes is lateral. The nystagmus is transitory, with the quick component to the normal side. The deviation of the eyes becomes maximal if the head is passively rotated to the normal side, but the nystagmus stops, and the rotation of the trunk ceases. The head and eye movements are direct effects of the extirpation of the labyrinth, while the neck and trunk reflexes are secondary and determined by the abnormal position of the head.

The rolling movements are only seen for a short time after the operation. Since the muscular tonus is diminished on the side of the lesion, the effect of any attempted progression movement is to cause the animal to turn over towards the injured side.

Bilateral Labyrinth Extirpation. The higher an animal is in the scale, the less important and the more readily dispensed with are its labyrinthine postural reflexes. Experiments on apes and cats show that, provided use of the eyes is retained, the animal behaves much as a normal one, except under two conditions.

The first of these is in falling free through the air. It is well known that a cat always falls on its feet, and this is accomplished by the employment

of ordinary postural reflexes, especially labyrinthine ones. During the fall, first the head is righted, then the fore limbs and thorax, finally the hinder part of the body. After bilateral labyrinthectomy this power of righting the body during a fall is lost.

The other case in which a response is lost is when the animal is placed in water. Under these circumstances the labyrinthectomized animal fails to orient itself, and would soon drown. The tonus of the muscles is not permanently lowered by removal of both labyrinths, but is kept up, as Sherrington has shown, by proprioceptive impulses from the muscles themselves, or from the muscles or other structures, such as joints, in other parts of the body, *e.g.* the neck.

The most usually accepted hypothesis is that the macular (otolith) organs are concerned with the *positions* of the head, while the ampullæ of the semicircular canals are concerned in notifying *movements*, particularly rotations. Whereas the former are static organs, the latter are kinetic.

Two views have been held regarding the causes of stimulation of the maculæ : (*a*) That bending of the hairs causes stimulation ; (*b*) that pulling on the hairs is the stimulus. As we shall see later, it is probable that the maximal stimulation is produced when the otolith organ hangs from and pulls upon, the hairs (A, Fig. 186), and the weakest stimulus when it pushes down vertically upon them (C, Fig. 186).

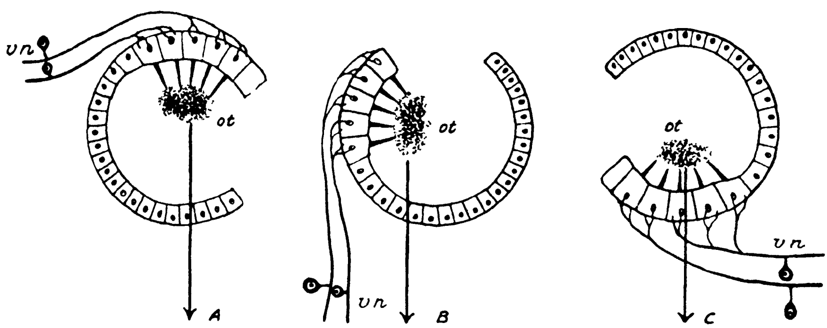

FIG. 186. Diagram to Illustrate the Influence of Position of the Head in Space on the Otolith Organ. In *A*, the otolith organ hangs vertically from the hairs, in *B* it pulls sideways, and in *C* it presses down on them. *v.n.*, fibres of the vestibular nerve. *ot.*, the otolith organ. The arrows indicate the pull of gravity.

Denervation of the utricle was found by Tait and McNally to cause loss of muscular tone on the same side of the body.

The functions of the macula of the saccule are not clear. Denervation seems to have no evident results. It is possibly an organ that responds to vibration.

The responses of the labyrinth to various stimuli have been investigated by Ross,* who used the severed head of the frog, and led off the auditory nerve. Responses were shown by action potentials in the nerve, and occurred when the preparation was subjected to tilting, angular acceleration, or vibration. The response to tilting was associated with the utricle macula, that to rotation with one of the canals, and that to vibration chiefly with the saccule macula. There seemed to be two receptors for gravity (tilting) and two for vibration.

Two views have similarly been held about the semicircular canals, but it is now considered that each canal operates in one direction of rotation only. Thus, on rotating the head about a vertical axis so that the nose

* Ross. *J. Physiol.*, 1936, **86**, 117.

moves to the left, there is stimulation of one horizontal canal (probably the right). If the nose moves to the right, the other horizontal canal is stimulated. Probably it is the bending of the hairs which is the essential factor. If the head is suddenly rotated about a vertical axis, the walls of the horizontal canals will rotate in the same direction. But the contained fluid remaining stationary owing to inertia will tend to apply forces to all structures with which it is in contact. The hairs in the ampullæ will then have a force applied to them tending to bend them, like reeds in a flowing stream. It has been objected that the canals have bores far too fine for any streaming of fluid to take place, but direct observation of the canals during rotation shows that movement does occur.

Tait and McNally * denervated one or more of the semicircular canals in the frog. When all the canals were denervated the animals assumed a normal posture, provided they were unmoved. Sudden tilting of the animal, however, failed to produce the normal compensatory movements of head and limbs. Their experiments show clearly that the horizontal canals only respond to rotations in the horizontal plane, while the vertical canals respond to rotation, each in its own plane. They infer that each horizontal canal is stimulated when moved in a direction away from its ampulla (' ampulla-trailing '), *e.g.* the left one only by turning the head to the right. The vertical ones, on the other hand, they believe to be stimulated when movement is such that the ampulla is foremost (' ampulla-leading '), *e.g.* the left anterior vertical by a movement of the head obliquely forwards and to the left (cf. Fig. 183). These results have been confirmed by Ross's direct recording of action potentials in the vestibular nerve.

Tonic movements of the musculature are produced when the cristæ of the canals are stimulated by suitable means, *e.g.* by rotations. The horizontal canals affect the musculature on the two sides of the body in opposite ways. When, for example, the whole animal is rotated to the right, the head itself turns to the left, the (extensor) tonus of the limbs on the right side is increased, and the tonus on the left side is reduced. The vertical canals each affect a corresponding quarter of the body; thus stimulation of the left anterior vertical canal causes increased tonus of the left fore-limb, that of the left posterior canal increased tonus of the left hind limb, and so on.

Stimulation of the Semicircular Canals in Man. This can be carried out by rotation, or by galvanic or caloric stimulation. The results of rotating a subject on a turn-table or rotating chair at about one revolution per second, are similar to those obtained with animals. They comprise nystagmus, vertigo, and post-rotatory reactions. Nystagmus is a compensatory movement of the head and/or eyes, and consists of a slow reflex component, and a jerky (cerebral) return. They are due to the fact that the gaze tends to remain fixed on some stationary point. Hence, if rotation is to right, the eyes (and sometimes the head) in keeping the gaze fixed, move to the left slowly, and when the limit has been reached, jerk rapidly back again to the right to recommence the next slow movement. This is called a right horizontal nystagmus, the indication being conventionally given to the *quick* component. Vertical nystagmus can also be produced by appropriate movements. After the rotation a nystagmus in the opposite direction occurs, due to inertia of the endolymph.

Vertigo, or giddiness is well known as a result of rotation. It is most marked after the rotation is over, and leads to compensatory movements in the opposite direction. Vertigo is a common clinical accompaniment of

* TAIT and MCNALLY. *Amer. J. Physiol.*, 1925, **75**, 140, 155; *Q. J. Exp. Physiol.*, 1933, **23**, 147; *Phil. Trans.*, 1934, **224B**, 241.

labyrinthine disorders. As a consequence of the tendency to fall over, various compensatory changes of muscular tone also occur after rotation, but are better seen after caloric stimulation.

Caloric stimulation (Barány's Test) is carried out by bending the head backwards at 60°, so as to bring the horizontal canals into a vertical plane, and then syringing the ear with cold water (20° C.). Vertigo and nausea result, and also definite after-effects, *e.g.* nystagmus to the opposite side, a curious twisting of the trunk, with extension of the arms, both of which are turned towards the side stimulated by cold (" discobolus position ") ; later the position is reversed. Another effect of the altered tonus is the 'Past-pointing Test '—on attempting to touch a given point with eyes closed, the arm is deviated towards the stimulated side.

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DECEREBRATE RIGIDITY

The state of decerebrate rigidity has already been mentioned as a release phenomenon, which is a caricature of the standing posture. The rigidity does not follow section of the medulla below the upper limit of Deiters'

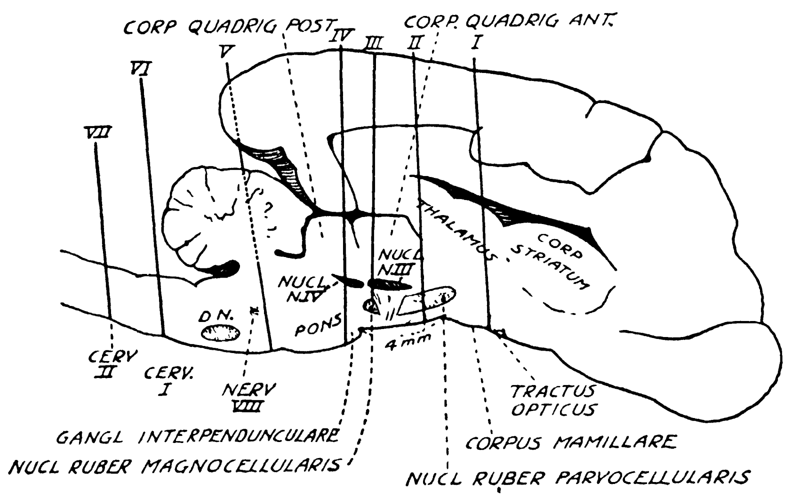

FIG 187. Longitudinal Section of Cat's Brain, showing the various sections of the brain stem in their relation to decerebrate rigidity.

Section I The Thalamus animal. Possesses heat regulation and normal postural reactions.

Section II. Mid-brain animal. Temperature regulation lost, posture normal

Section III, below red nucleus. Decerebrate rigidity.

Section IV, decerebrate rigidity present.

Section V, just above vestibular nuclei: decerebrate rigidity.

Section VI, below vestibular nuclei (D N.); no rigidity. Neck reflexes still present.

Section VII, spinal animal. No neck reflexes. (Re-drawn, after Magnus.)

* FULTON, LIDDELL and RIDDOCH. *Brain*, 1930, **53**, 327.

nucleus; thus, Section VI. of Fig. 187 does not produce it, but Section V. does; hemi-section causes rigidity on the same side only. The rigidity is at once diminished or abolished if the vestibular nuclei (including Deiters') are destroyed,* or if the ventro-lateral columns of the cord are divided. These facts seem to indicate the vestibular nuclei, and the vestibulo-spinal tract which descends from them, as essential for the condition, and suggest that this nucleus normally exercises on the extensor muscles a tonic influence, which is here exaggerated by release from the modifying influence of some centres still higher up.

The location of these higher centres is ascertained by making sections from before backwards. It will be seen from Fig. 187 that rigidity does not then appear until the section passes through the mid-brain, just below the red nucleus, or through its anterior part. It is inferred, therefore, that the centres which in the normal condition keep the vestibular nuclei from over-acting are at the level of the red nuclei, but it does not necessarily follow that the red nucleus is the only centre concerned. Doubtless it is one of them, but destruction of the red nucleus alone does not cause rigidity.* Nor is it certain that section of the rubro-spinal tract alone will cause rigidity. Some believe that the hypothalamus contains another similar centre.

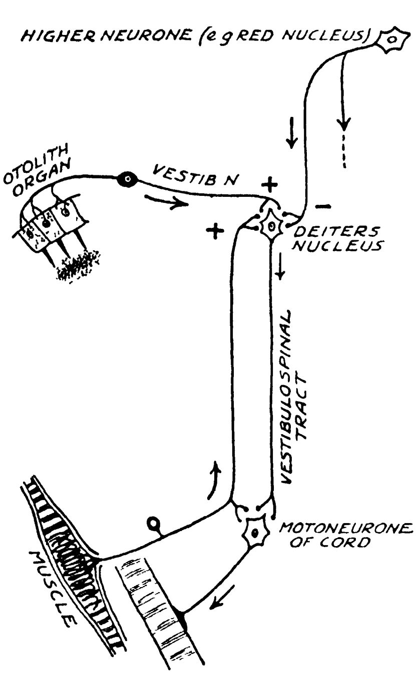

FIG. 188. Diagram to Illustrate the paths concerned in Postural Tonus and Decerebrate Rigidity.

The rigidity is of reflex origin, the afferent impressions concerned arising from the muscles themselves, and from the labyrinth, and being in both cases relayed from the vestibular nuclei. The afferents concerned in the rigidity of the hind limbs come from the muscles only; hence, division of the afferent roots of the muscular nerves abolishes it, while removal of the skin does not. For the fore limbs, de-afferentation does not remove the rigidity, but destruction of the labyrinths as well will do so.†

These relations are shown diagrammatically in Fig. 188.

The muscles which become tonic in decerebrate rigidity are those which counteract the effects of gravity, in maintaining the normal posture of the animal. These are, in most animals, the extensor muscles. In the sloth, which hangs upside down, the flexor muscles are the tonic ones.

Decerebrate Rigidity in Man. A condition closely resembling decerebrate rigidity is also seen in the human subject as a result of certain lesions of the brain stem, *e.g.* the result of suprapituitary tumours. The subject shows extension of the legs, with the feet pointed down in plantar flexion; the

* INGRAM and RANSON. *Amer. J. Physiol.*, 1932, **102**, 466.
† POLLOCK and DAVIS. *Amer. J. Physiol.*, 1931, **98**, 47.

arms are semi-flexed at elbows, forearm pronated, and wrists and fingers flexed * (Fig. 189).

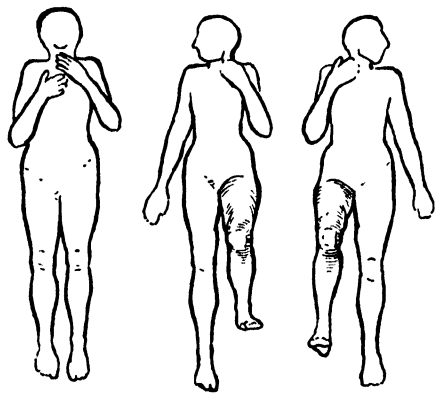

FIG. 189. Decerebrate Rigidity in Man. SAMSON WRIGHT, after WALSHE, "Applied Physiology," Oxford University Press.

Rotation of the head causes extension of the arm on the side to which the nose is turned, and flexion, with augmented Babinski response, of the opposite leg (Fig. 189). This is a postural reflex, which is explained later.

THE POSTURAL REFLEXES

The higher animals normally exhibit characteristic attitudes in repose or action, by which they are oriented with reference to gravity. To any circumstance which tends to displace the animal from its posture there is offered a resistance which prevents the displacement, or which tends to restore the animal to its correct attitude.

Static postures are often maintained for long periods of time without fatigue. The state is one of tonic contraction of the muscles concerned, and the assumption of any posture is the result of a delicate condition of balance in the muscular system. In the maintenance or recovery of posture many separate types of reflex converge to produce a common result; these reflexes arise from the labyrinth, the eyes, and the muscles themselves.

The removal of the cerebral hemispheres has but little effect on normal posture, or on the righting reactions by which that posture is resumed after having been interfered with. It is clear, therefore, that these reactions are true reflexes. In a fully conscious animal, of course, voluntary resistance may be added to the reflex reaction.

Some muscular tonus persists in the spinal animal (Fig. 190B), but this is slight and bears no resemblance to normal posture. The bulbo-spinal animal, by contrast, shows the condition of *decerebrate rigidity* (Fig. 190A), which is due to an exaggerated state of tonus of those muscles (extensors) which are employed in the posture of standing. Such a decerebrated animal can, in fact, maintain a standing posture, but when upset it makes no movements which would tend to replace it in its former standing pose. It was shown by Sherrington that the tonus distribution in the decerebrate preparation is not entirely proprioceptive, but depends, for instance, on the position of the head.

* WALSHE. *Lancet*, 1923, **101** (ii), 644.

When the whole of the mid-brain is left as well, the attitude of standing is normal (Fig. 190C), and, moreover, the animal will now of its own accord return to the standing posture when displaced from it. Most of the postural reflexes of the 'thalamus' animal (*i.e.* with intact thalamus and mid-brain) are practically identical with those of the normal animal. (The advantage of leaving the thalamus is that temperature regulation is retained.) This fact, and the knowledge which we derive from our own experience, teach us that the afferent impulse upon which posture is based and adjusted but rarely obtrude into the field of consciousness, and can, therefore, only be studied by objective methods. Such studies have been made by Magnus, and to translate his own words freely, have shown "that many different sense organs are involved, that very different centres integrate their stimuli, that the muscles are concerned in many different combinations in the production of the reactions, and that a whole series of diverse reflex groups are balanced together."

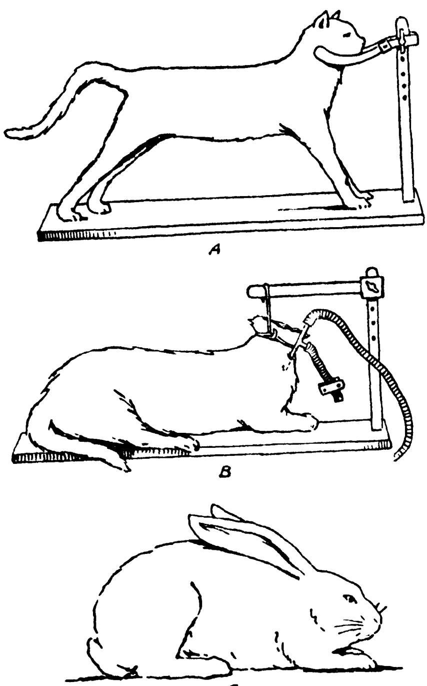

FIG. 190 Diagram illustrating the effect on Posture of Ablation of different Parts of the Central Nervous System *A*, Section of mid-brain Decerebrate rigidity *B*, Section of upper spinal cord (decapitate animal) Loss of muscular tone. *C*, Removal of cerebral cortex and basal ganglia in front of thalamus Normal posture and righting reflexes'

The postural reactions of the body when at rest are called *static reflexes*, and fall into two groups: the *stance reflexes* or reflexes of *pose*, in which the body, or a part, remains in a definite attitude, and *righting reflexes*, by means of which the pose is restored after disturbance. The former, so to speak, 'keep you right way up when you are' while the latter 'put you right way up when you are not.'

The postural reactions of the body when in movement are called *stato-kinetic reflexes*, and are produced in a definitely predictable manner by the movement itself, which in turn is largely guided by them under normal conditions of life.

These important postural reflexes fall into the following heads and it will be noted that there is a good deal of inter-dependence.

I. Static Reflexes

(i) *Local Static Reactions*, *e.g.* confined to one limb.
(ii) *Segmental Static Reactions*, involving one segment, *e.g.* both hind limbs, both fore limbs, or the neck.
(iii) *General Static Reactions*, involving more than one segment. These may be again divided into :—

A. *Reflex Stance.*
   (1) Influence of the Head on Stance.
      (a) Effect of Tonic Neck Reflexes on Limbs.
      (b) Effect of Tonic Labyrinth Reflexes.
         (α) On the Limbs.
         (β) On Neck and Trunk.
      (c) Collaboration of Neck and Labyrinth Reflexes.
      (d) Indirect Influence of Labyrinth on Limbs through Action on Neck Reflexes.
   (2) Indirect Influences on Posture.

B. *Compensatory Pose of Eyes.*
   (1) Effect of Tonic Labyrinth Reflexes on Eyes.
   (2) Effect of Tonic Neck Reflexes on Eyes.
   (3) Collaboration of Tonic Labyrinth and Neck Reflexes on Eyes.
   (4) Collaboration of Compensatory Eye Poses.

C. *Righting Reflexes.*
   (1) Effect of Labyrinth Reflexes on the Head.
   (2) Effect of Body Posture on the Head.
   (3) Neck Postural Reflexes.
   (4) Effect of Body Postural Reflexes on the Body itself.
   (5) Visual Postural Reflexes.

II. Stato-kinetic Reflexes

A. *Reactions to Rotation.*
   (1) Head Reactions.
   (2) Eye Reactions.

B. *Reactions to Progressive Movement.*
   (1) Reactions of Head.
   (2) Reactions of Extremities.

We must now consider these types.

I. STATIC REFLEXES

(1) Local Static Reactions

It is a remarkable fact that a limb which at one time is freely moveable, at another time is converted into a rigid pillar capable of supporting the weight of the body. The formation of the rigid support by simultaneous contraction of flexors and extensors is due to a local reflex, called by Magnus the *positive supporting reaction*, while its resolution, enabling the limb to be used for other purposes, is also a local reflex, called the *negative supporting reaction*. These reflexes receive their explanation from the researches of Sherrington and Liddell, and of Magnus and his collaborators, Rademaker, Schoen and Blake Pritchard.

The afferent stimuli for the positive supporting reflex are in part proprioceptive stimuli evoked by movements of the distal parts of the limbs, by which some of the flexor muscles are stretched, and in part are due to exteroceptive stimuli from the skin, *e.g.* contact of pad of foot with the ground. A direct consequence of the stretching is that these flexor muscles themselves are reflexly contracted (stretch reflex) ; a second result is that there is reflex contraction of flexors and extensors, whether stretched or not,

largely by reason of a reciprocal innervation effect, the antagonistic muscles thus acting synergically in fixation, at the proper angle, of the joints concerned. In this complex local reaction very different reflexes co-operate, reinforce, and if necessary replace each other, so that standing under very different positions of the joints is possible.

In the negative supporting reaction, the active stimulus is proprioceptive, due to volar or plantar flexion which unlocks the series of positive reflexes and loosens the limb for action.

(ii) SEGMENTAL STATIC REACTIONS

A good example of these is the crossed extension reflex already studied.

(iii) GENERAL STATIC REACTIONS

A. REFLEX STANCE. (1) *Influence of the Position of the Head.* Experiments on the effect of the position of the head on the tonic pose of the body are carried out on decerebrate animals. The distribution of tonus is modified as a result of stimuli from the neck when the head is moved with reference to the trunk, and also, by the operation of labyrinthine reactions, when the head (with the body) has its position altered in space. These two effects can be separated experimentally.

(*a*) *Tonic Neck Reflexes.* These are obtained in a pure form on animals deprived of both labyrinths, and are also seen in certain clinical cases. When the head is moved by bending or twisting the neck, the extensor tonus of the decerebrate preparation shows modifications which persist so long as the altered relation of head to trunk is kept up. These alterations are due to altered tensions on the neck muscles when the head is moved. The muscles of the right and left sides or of the fore and hind limbs behave in opposite ways.

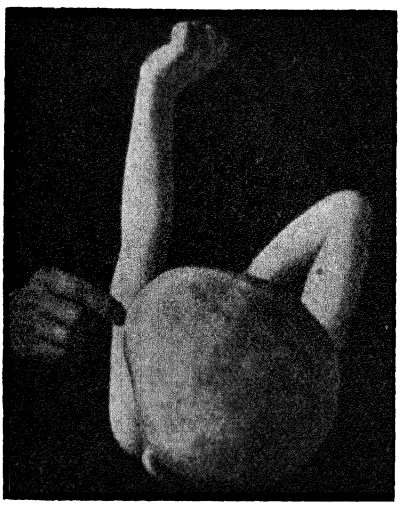

FIG. 191. Hydrocephalic infant. Turning of head to left gives increased tonus of left arm, due to tonic neck reflex. (MAGNUS, "Korperstellung.")

When the head is rotated on the atlanto-epistropheal joint, the extensor tonus of the fore and hind limbs is increased on the side towards which the nose is turned, and diminished on the other side (Fig. 191).

When the head is inclined sideways by bending the neck, so that, say, the left ear approaches the left shoulder, the extensor tonus of the limbs is increased on that side, while on the right side there is a diminution. This reaction to bending of the neck obviously serves the purpose of keeping the centre of gravity of the body over the feet.

Bending the neck backwards causes increased tonus of the fore limbs, with reduction of tone of the hind limbs. This results in a posture such as is seen in a normal cat craning the neck upwards to look on a table. When the head is bent downwards, the tonus distribution is in the opposite direction, as in a normal cat eating food.

The centre which is responsible for these neck reactions is located in the upper cervical spinal cord and lower medulla.

(*b*) *Tonic Labyrinth Reflexes.* These reflexes are best studied on decerebrate cats with intact labyrinths, and fixed to a holder in such a manner that the head cannot be moved on the trunk.

The most conspicuous effect is that on the limbs, all of which react similarly. Maximal extensor limb tonus is seen when the animal is placed on its back, so that the mouth cleft is inclined upwards at 45 degrees to the horizontal; when the animal is placed on its belly so that the mouth cleft is downwards at 45 degrees, the extensor tonus falls to a minimum. At intermediate positions the tonus is of intermediate intensity.

The receptors concerned in these reflexes are the maculæ, chiefly those of the utricles. The utricle macula lies in a horizontal position when the head is normally oriented, and the otolith then lies above the hair cells which spring from the macula. The macula of the saccule is situated on the lateral wall, and is, therefore, vertical when the head is normally placed,

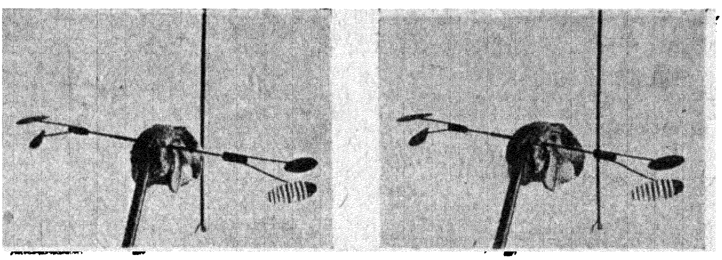

FIG. 192. Rabbit's Skull with Oriented Magnified Models of the Utricle and Saccule Maculæ. Skull in normal position Stereoscopic. (MAGNUS, "Korperstellung.")

the hairs of its hair cells lying horizontally in the otolith. The exact orientation of these maculæ in the rabbit is illustrated in Fig. 192, which shows a rabbit's skull in the normal position in space, with enlarged models of the maculæ, represented by oval plates, in their proper spatial arrangement, attached to it. The surface of the macula to which the otolith is attached is indicated in the utricle by white dots, and in the saccule by white stripes.

When the animal is placed in the position which gives the maximal labyrinth tonus it is found that the utricle otolith is hanging vertically beneath its macula; when the head is placed in the position which gives the minimum of limb tonus, then it is found that the otolith is vertically over its macula. Hence it is concluded that the otolith produces the greatest stimulus when it hangs from, and so pulls upon, its hair cells, and the least when it presses them downwards on to the macula. The stimuli are continual, and give rise to steady reflex tonus so long as the position is kept up. Similarly, the saccule macula is most stimulated when the otolith hangs from it, as on the left side when the left side of the head is below. Hence there arise, from the asymmetric nature of the stimuli from the two saccules, asymmetric states of tonus, which constitute the righting reflexes of labyrinthine origin.

Deprivation of both labyrinths leads to complete disappearance of the reactions to altered orientation.

The tonic neck and labyrinth reflexes show an algebraic summation in the normal animal, in which they both have full play. It is also found

that the labyrinth exerts an indirect action by giving rise first to tonus changes in the neck, which produce neck movements, and hence, by the operation of the neck reflexes, exert an effect on the limbs. The two effects of the labyrinth on the body are algebraically summated.

In these postural reflexes the red muscles play a larger part than the pale muscles (Fig. 193).

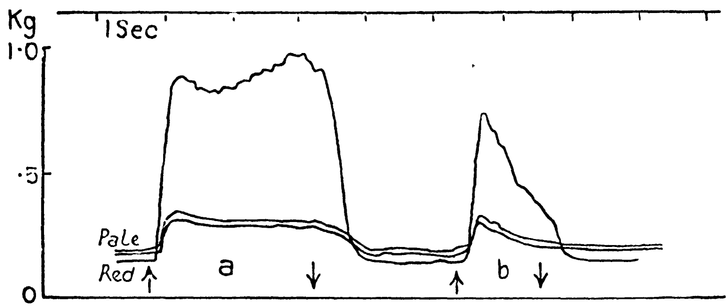

FIG. 193. Response of Pale (Short Lat Head Triceps) and Red (Short Med. Head Triceps) Muscle to Labyrinthine and Neck Reflexes Mid-brain preparation ↑ dorsiflexion and ↓ ventriflexion of neck Tracing from pale muscle shown by double line and from red muscle by single line CREED, *et. al* "Reflex Activity in the Spinal Cord" Oxford Univ. Press, 1932)

(2) *Indirect Influences on Posture.* Posture in the normal animal is largely influenced by impressions received from the outer world through the senses of sight and hearing. These lead to voluntary movements of the head and, by the operation of the reflexes so far described, to an automatic modification of the pose. To take the cat as an example, we have already referred to certain characteristic postures related to neck reflexes, and a little consideration will show that the pose will be further reinforced by the action of the labyrinth reflexes.

Suppose that a cat hears a mouse moving on its right. The head is turned to the right; this alters the centre of gravity, but, as a result of the tonic neck reflex, there is an increase of tonus in the muscles on the right side of the body, which preserves the balance by throwing the weight on the right limbs. Now, because the left limbs are less loaded, they will be the first to move if the animal springs to its prey, so that the cat will automatically move in the right direction.

B. COMPENSATORY POSE OF THE EYES. The compensatory pose exhibited by the eyes under certain conditions is really a special case of stance reflexes. In man and other animals with frontal vision it is of much less importance than in such animals as the rabbit, which have lateral vision. Like other muscles, the tonus of the eye muscles may be modified as a result of reflexes arising either from the labyrinth or from the neck. The outcome is that the eyes take up a position such that their visual fields suffer as little change as possible.

(1) *Tonic Labyrinth Reflexes on the Eyes* In these reflexes no effect is produced by way of the labyrinth unless movements alter the relation of the head to the horizontal plane. The responses are to rotation of the head (and body) about the occipito-nasal axis ('vertical' response) and rotation around the bi-temporal axis ('rotatory' response). In the performance of these movements there is always a delicately adjusted reciprocal action of antagonistic muscles in such a manner that the eyes continue, as it were, to look at the same object in spite of the movements of

the animal. Thus if the animal in the prone position is tilted so that the head moves downwards, the eyes will move upwards, and *vice versâ*.

The centres for all labyrinth reflexes on the eyes lie between the vestibular nerve entry and the nuclei for the eye muscles.

(2) *Tonic Neck Reflexes on the Eyes.* In these reactions, which are obtained after destruction of the labyrinth, when the head is moved with relation to the body, *all* the eye muscles are brought into play, and compensatory eye poses are obtained in response to alteration of the head with reference to any plane, including the horizontal. The effects are similar to those obtained with the labyrinth reflexes.

Under normal conditions the labyrinth and the neck reflexes are both present and are summated algebraically, so that, while neither reflex alone is enough to compensate fully for head movements, the addition of the two does so. The exception to this is when the movement of the head is in the horizontal plane, in which case, as already stated, there is no labyrinthine reflex set in operation.

C. RIGHTING REFLEXES. The reactions by which an animal keeps itself right side up and recovers its normal posture after it has been disturbed, are seen best in thalamus animals, so that voluntary movements are eliminated. An animal takes up by means of these reactions a normal stance, which is the basis for other voluntary or reflex movements. Righting reflexes are present at birth, and are carried out by co-operation of five different groups of reflexes. When, by suitable procedure all of these are eliminated, we have the '*zero condition*,' in which no righting reflexes are shown. In rabbits this is achieved by destruction of both labyrinths, and holding the animals free in the air ; cats, dogs and monkeys, which use the eyes for righting reactions must also be blindfolded if the cerebrum is intact. Knowing the zero conditions we can compare the effect of introducing the responsible reflexes one by one.

(1) *Labyrinthine Righting Reflexes and Head Pose.* As a result of these reflexes, the head tends to return to its normal position in space.

When a thalamus rabbit is held up in the air by gripping the pelvis (to avoid contact stimuli which would be introduced if the animal were allowed

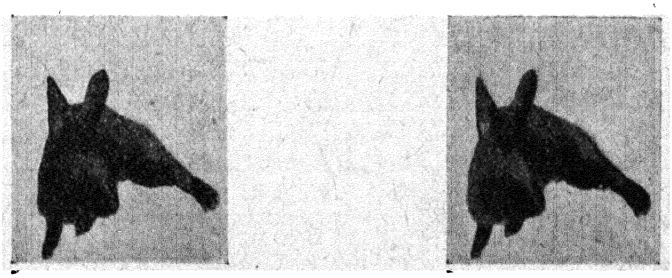

FIG. 194 Response of Thalamus Rabbit to Rotation of Pelvis when held Free in Air. The anterior part of the body retains the normal position owing to co-operation of labyrinth, and neck-righting reflexes. View with stereoscope (MAGNUS, "Korperstellung.")

to lie on the ground), it is seen that the head tends to remain in the normal position when the pelvis is moved to the most various positions. This is accomplished by appropriate twisting or bending of the vertebral column (Fig. 194). After extirpation of both labyrinths the reflexes are abolished (Fig. 195).

(2) *Body Posture on the Head.* If a rabbit deprived of both labyrinths be laid on the ground, the head again tends to assume the normal pose. This

is due to the operation of asymmetric stimuli arising from contact of only the lower side with the ground. Thus, if the animal is laid on its right side, contact stimuli will arise from that side, and not from the left, and these

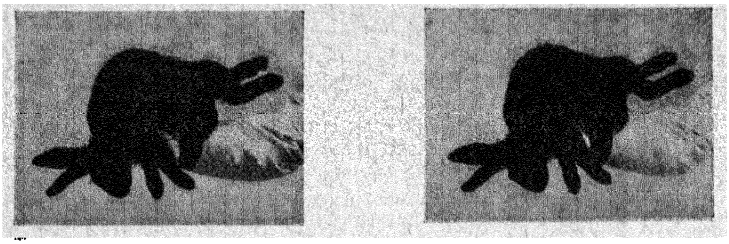

FIG. 195. Thalamus Rabbit ('Zero Condition') after Deprivation of Labyrinths, for Comparison with Preceding Figure. Stereoscopic (MAGNUS, "Korperstellung.")

lead to orienting reactions of the head (Fig. 196). If the stimuli are made symmetrical, as by placing the animal in the lateral position and laying a board on its upper side, the head orientation vanishes (Fig. 197).

FIG. 196. Thalamus Rabbit without Labyrinths laid on Right Side The head and then the anterior half of the body, assumes the normal pose owing to asymmetric contact stimuli. Stereoscopic. (MAGNUS, "Korperstellung.")

Owing to these two reflexes from labyrinth and from contact stimuli, there results a twisting of the neck when the animal is displaced, and this, in its turn, leads to the production of

(3) *Neck Reflexes.* These operate in much the same way as the tonic neck reflexes, and the result of the combination of all these reflexes is that first the front and then the hind limbs follow the posture taken by the head,

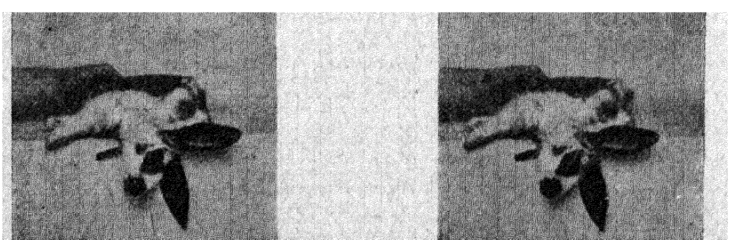

FIG. 197. The same Rabbit as last Figure laid on Side, but with Weighted Board placed on Upper Side, no Righting Reaction. Stereoscopic (MAGNUS, "Korperstellung")

so that ultimately the whole animal comes to resume its normal pose. For instance, suppose an animal is laid on its left side. In consequence of labyrinthine and contact reactions, the head is turned towards the left

shoulder (*i.e.*, remains horizontal); this leads to neck reflexes and increase of tonus of the left fore limb, which rights the fore part of the body, then to increase of tonus in the left hind limb, by which the hind part of the body is also righted.

(4) *Reflexes from the Trunk upon the Body itself.* The effect of asymmetric contact stimuli such as arise when an animal is laid in the lateral position affects not only the head, but also the body itself, so that if the head is fixed in the lateral position the body may be righted even though this entails opposition to the neck and labyrinth reflexes. In the normal animal there is thus a double reflex mechanism, which is summated to produce the total effect.

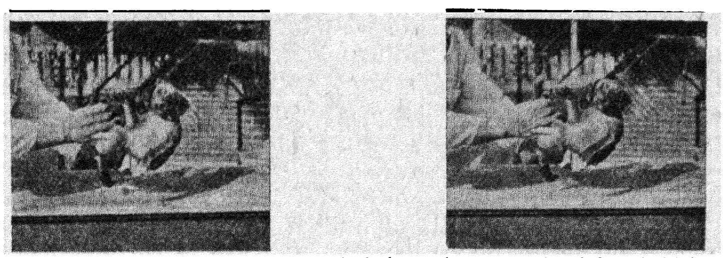

FIG. 198. Dog Sixteen Days after Double Labyrinthectomy, the Pelvis held in the Air in Horizontal Dorsal Position; Righting of Head and Thorax. Stereoscopic. (MAGNUS, "Korperstellung.")

(5) *Visual Righting Reflexes.* These reactions can be studied in cats and dogs with intact cerebral cortex after bilateral labyrinthectomy. The animals can orient their heads quite well if allowed to use the eyes, but when

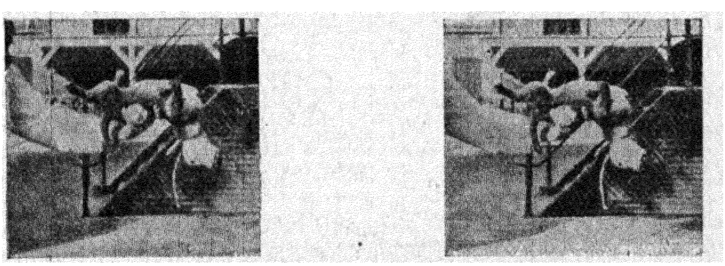

FIG. 199. The Same Dog held in the same Manner, but with the Eyes Covered; no Righting Reaction. Stereoscopic. (MAGNUS, "Korperstellung.")

these are covered they revert to the disoriented position of the head, just as the labyrinthectomized rabbit does (Figs. 198 and 199).

Centres for Righting Reflexes. Visual righting reflexes are cortical. It has been shown by Rademaker, using the method of progressive section at various levels, that the other centres responsible for the righting reflexes are situated in the mid-brain and pons. The neck-righting reflexes are located in the pons, the remainder are in the ventral portion of the mid-brain at about the level of the third nerve nucleus, and probably in the large-celled portion of the red nucleus from which the rubrospinal tract arises. Destruction of the pyramidal tract, substantia nigra, or cerebellum leaves the righting reactions unchanged. Mesial sagittal section of the mid-brain cause disappearance of righting reflexes if Forel's decussation has been divided, not otherwise.

The spinal centres appear to be under the influence of higher centres of two types: medullary centres, which tend to cause over-action of the extensor muscles, and the red nucleus, which, acting *viâ* the rubrospinal tract, tends to counteract this by throwing the flexors into action. By the combined action of the two we get in the thalamus

animal a normal distribution of tonus; the pyramidal tracts in the intact animal also exert a flexor effect on the spinal centres.

II. STATO-KINETIC REFLEXES

Unlike the righting reflexes, these are produced by the actual movement, and not by any abnormal orientation. In so far as they arise from the labyrinth, they may be said to be due not to movement in itself, but to an acceleration, positive or negative. This may be either angular acceleration, *i.e.* movements of rotation, or progressive acceleration when the movement is in a straight line.

A. REACTIONS TO ROTATION. (1) *Reactions of the Head.* When an animal or a man is rotated, say, to his right, the eyes tend to remain fixed on a given point, and hence the head is steadily moved to the left as far as possible, and then sharply to the right, this movement being repeated. This is called *head nystagmus*, with the rapid component in the same direction as that of the rotation. After the rotation has been stopped the nystagmus is in the opposite direction, *i.e.* in the case supposed, with the rapid component to the left.

All these phenomena may be explained as due to the effects of the rotation on the semicircular canals.

(2) *Eye Reactions.* The reactions of the eyes to rotation are similar to those of the head: that is to say, eye nystagmus, in the same direction as the rotation, is produced. The effect on the eyes is naturally best seen when the head is fixed with relation to the body. On cessation of rotation the nystagmus is reversed.

When the head instead of the whole body is moved, the response of the eyes to the movement of rotation so produced leads to their moving in a direction towards that in which they finally become fixed by the static reflexes already considered.

B. REACTIONS TO PROGRESSION MOVEMENTS. (1) *On the Head.* These are best seen in the dog when stood on a board. When moved suddenly upwards, the head is moved ventrally and the fore-paws flexed; when the movement ceases, the head moves dorsally, and the fore-paws are extended. On movement downwards the opposite effects are produced.

(2) *On the Extremities.* When the animal is held head downwards, the reaction to sudden downward movement is that the fore-limbs are moved in an oral direction, as in preparing the animal to take up its weight on falling to the ground.

C. REACTION TO MOVEMENTS OF PARTS OF BODY. When a part of the body is moved reflex adjustment of pose is often necessitated to maintain the balance, and the responses described above are variously brought into play. Thus, if a dog has an ulcerated foot, that leg is held in a position of flexion, and the posture and the movements of locomotion are arranged in accordance with the modified support which the body now has. This is all reflex, and depends on the constant arrival, at the centres concerned, of stimuli from the affected foot.

In general we can say that by means of the stato-kinetic reflexes the body is brought into its normal stance, which pose is maintained by the operation of the various static reflexes. It will have been noticed that the head plays an important part in deciding most of these responses. The head contains the organs of special sense, particularly the distance receptors for sight and sound, so that the head tends to be voluntarily directed towards stimuli which affect these sense organs. The proper orientation of the

remainder of the body which ensues is effected by the action of the various reflexes which have been described.

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CHAPTER XIX

HIGHER AFFERENT PATHS

MANY of the afferent fibres of the spinal cord, in addition to furnishing collaterals which subserve the purposes of spinal reflexes, ultimately ascend to higher levels. Some after various relays ultimately reach the cerebrum, and convey impressions to consciousness ; others establish connections with higher reflex centres ; some do both. These various paths must now be briefly considered, together with those of the more specialised senses. Usually three neurones, primary, secondary and tertiary (or first, second and third order), are met with in the course of an afferent path from periphery to the highest centre, but auditory and visual paths have more.

I. CONSCIOUS SENSATIONS

So far as the brain stem is concerned, the main afferent tracts *en route* for the cerebral cortex are grouped together as the fillet tracts or lemnisci. These are the *mesial lemniscus*, *spinal lemniscus* and *trigeminal lemniscus*, all consisting of secondary neurones concerned with general body sensations, which end at the thalamus (on the side of the body opposite to that on which the primary neurones originate) ; and the *lateral lemniscus*, the afferent path for hearing, which consists of secondary neurones terminating in the medial geniculate body and inferior colliculus. Third (or higher) order neurones complete the path from thalamus (or other intermediate station) to the cortex.

(1) TACTILE AND KINÆSTHETIC SENSATIONS. (*a*) *The mesial lemniscus* represents the continuation of the *dorsal columns* of the cord, and therefore conveys conscious kinæsthetic and fine tactile impulses. These primary fibres end at the *nuclei Gracilis and Cuneatus* in the lower medulla, the ones from lower limbs at Gracilis, those from upper parts at Cuneatus (Fig. 200). The cells of these two nuclei give off secondary axons which cross the midline and pass upwards in the mesial lemniscus through pons and mid-brain and posterior limb of the internal capsule, to reach the lateral nucleus of the thalamus. From this tertiary fibres pass mainly to the sensory cerebral cortex (but some to the mesial and ventral nuclei of the thalamus).

(*b*) *The spinal lemniscus* is formed from the *two spinothalamic tracts*. The primary neurones of this path end in the grey matter of the cord, and its secondary axons have already crossed in the cord. The fibres of the *lateral spinothalamic* tract, conveying pain and thermal sense, remain in a superficial position on the lateral aspect of the medulla, while those of the *ventral spinothalamic* tract, mediating light tactile sensations, lie more deeply. Ultimately both run with the mesial fillet and terminate in the lateral nucleus of the thalamus, from which third order fibres proceed to the cerebral cortex (or mesial and ventral thalamic nuclei).

(*c*) *The trigeminal lemniscus* is derived from the sensory root of the Vth cranial nerve, the cell station being in the Gasserian ganglion. These primary neurones branch, forming ascending and descending 'roots.' These end in the sensory trigeminal nuclei, in pons, medulla and cervical

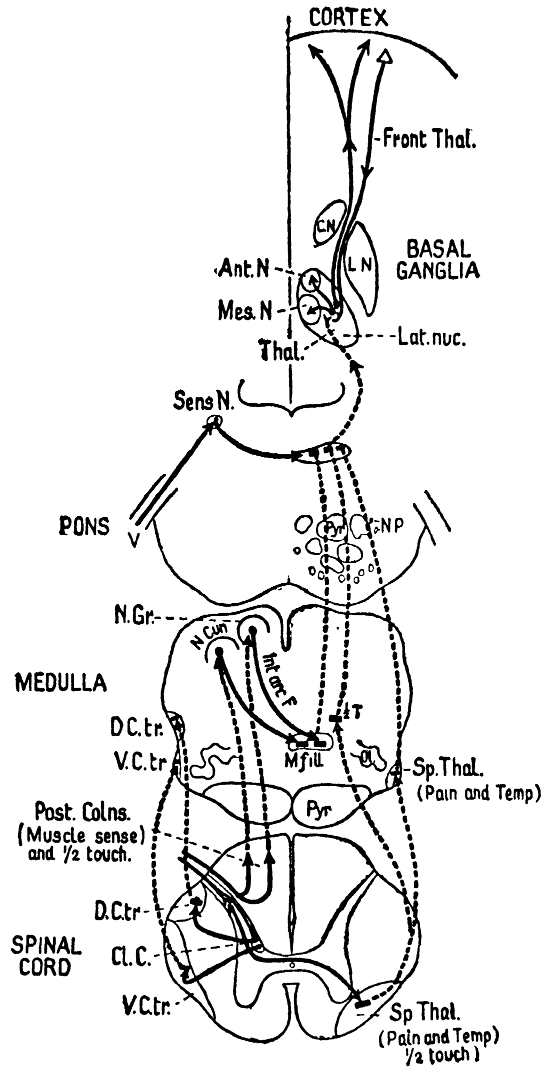

FIG. 200. Diagram of Course of Sensory Impulses Front Thal = Fronto-thalamic fibres; C.N. = Caudate Nucleus; L N. = Lenticular nucleus; Ant. N. = Anterior nucleus; Mes. N. = Mesial nucleus; Lat nuc = Lateral nucleus; (of Thal. = Thalamus) V. = Afferent fibres of Vth nerve; Sens. N. = Sensory nucleus of Vth nerve; N.P. = Nuclei pontis, Pyr. = Pyramidal tract; N Gr. = Nucleus gracilis; N. Cun. = Nucleus cuneatus; D C. tr. = Dorsal spino-cerebellar tract; V.C. tr. = Ventral spino-cerebellar tract; Int arc. F. = Internal arcuate fibres; M. fill. = Mesial fillet; Ol = Olivary nucleus. Sp Thal = Spino-thalamic tract; ½T = Half of touch fibres which crossed in the spinal cord; Cl. C. = Clarke's column. (SAMSON WRIGHT, "Applied Physiology," Oxford University Press.)

cord, and from the cells of these arise the second order fibres which cross the midline at various levels and proceed with the two other lemnisci to the thalamus.

Afferent fibres of the VIIth, VIIIth (vestibular), IXth and Xth cranial nerves also join the common fillet in a similar manner. Of the vagus fibres, for instance, about 70 per cent. are afferent, mostly unmyelinated, and with

cell stations chiefly in the ganglion nodosum * ; it has many important reflex functions.

(2) AUDITORY SENSATIONS. *The lateral lemniscus* is the path for auditory impulses. These arise in the cochlea and pass in by primary axons, with cells in the spiral ganglion, to form the cochlear division of the VIIIth cranial nerve. This divides round the restiform body to end in either the dorsal or ventral cochlear nuclei.

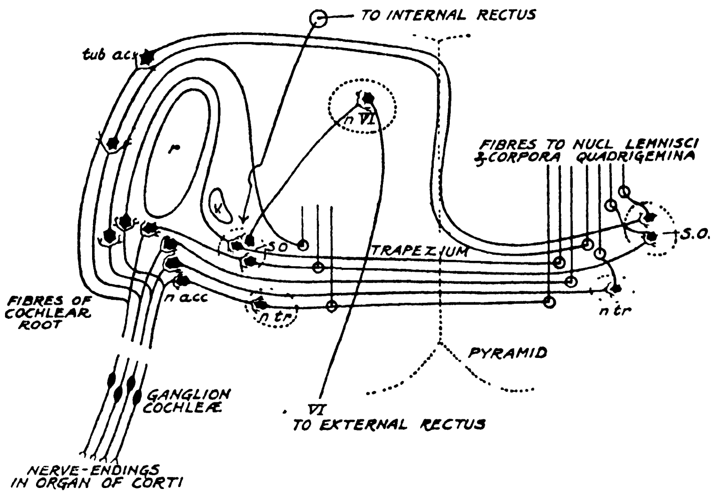

FIG 201 Connections of Cochlear Nerve r, Restiform body, *tub. ac*, Dorsal cochlear nucleus, *n acc*, Ventral cochlear nucleus; *s o*, Superior olive; *n tr*, Nucleus of trapezium; *n* VI, Nucleus of VIth nerve. (SCHAFER)

From these nuclei, secondary fibres pass to a second relay station, either in the superior olive or in the nuclei of the trapezium, as shown in Fig. 201, ultimately reaching, by third order fibres, the lateral fillet of either the opposite, *or of the same*, side. The lateral fillet passes up into the mid-brain, lying on its lateral aspects whence its fibres either turn inwards to terminate in the grey matter of the inferior colliculus, or pass on to reach and end in the medial geniculate body The medial geniculate body is the relay station connected with auditory sensations. The two medial geniculate bodies are connected together by Gudden's commissure; so that both ears are represented, directly or indirectly, on each side. From the mesial geniculate bodies the auditory radiations convey the impulses to the cortex in the temporal lobe. It is claimed † that small localized lesions in the medial geniculate body lead to loss of appreciation of particular pitches of sound, *e.g.* in the dorsal part to loss of high notes and in the ventral part to loss of low-pitched notes This would mean that particular fibres carry impulses aroused in the cochlea by particular notes.

Auditory Reflexes. The inferior colliculus acts as a centre for auditory reflexes; from it arise the tecto-bulbar, tecto-spinal and other tracts, such as the posterior longitudinal bundle, by which auditory impressions are correlated with movements of head and eyes. Another important connection of the cochlear nerve is one *viâ* the superior olive to the oculomotor nuclei, and one direct to the VIth cranial nerve (Fig. 201) which is motor to the external rectus muscle. Hence on hearing a sound, the eye is reflexly turned to that side. In animals the incidence of sound on the ears

* FOLEY and DU BOIS. *J. Comp. Neurol*, 1937, **67**, 49.
† ADES, METTLER and CULLER. *Amer. J Physiol.*, 1939, **125**, 15.

causes erection of the pinnæ. This is effected by a reflex, the ingoing path of which consists of auditory nerve fibres, and the outcoming path of fibres of the VIIth nerve. Another auditory reflex is the contraction of the tensor tympani and stapedius muscles when sounds first fall on the ear ; these muscles are innervated by a branch of the Vth cranial nerve. This reflex is bilateral even if sounds fall on one ear only It is most marked for sounds of high pitch. Its object is to put the drum into correct tension for the perception of sounds.

Other reflexes are protective. According to the strength of the stimulus there may be blinking of the eyes only, or, if the sound is louder, blinking and holding the breath. If stronger still, in addition to the above, all movements temporarily cease, and for a very loud sound indeed the limbs may become toneless and the body may fall. The details of this reflex arc are not known. The superior olive may be the relay centre fo. impulses to the Xth cranial nerve in holding the breath.

(3) VISUAL SENSATIONS. The rods and cones of the retina are visual sensory end-organs. The bipolar cells of the retina would then be the first order relay neurones, and convey the impulses to the second order neurones, or ganglion cells. The axons of these pass out from the eye as the optic nerve. Having entered the cranial cavity the nerve meets its fellow from the other eye, forming the optic chiasma, in which fibres partially decussate. The fibres thus form the optic tracts which travel round the crura cerebri and then divide to end in relation to neurones in three different places, viz the pretectal region (a transitional area between thalamus and mid-brain), the superior colliculi, and the lateral geniculate body (Fig 202). The first two

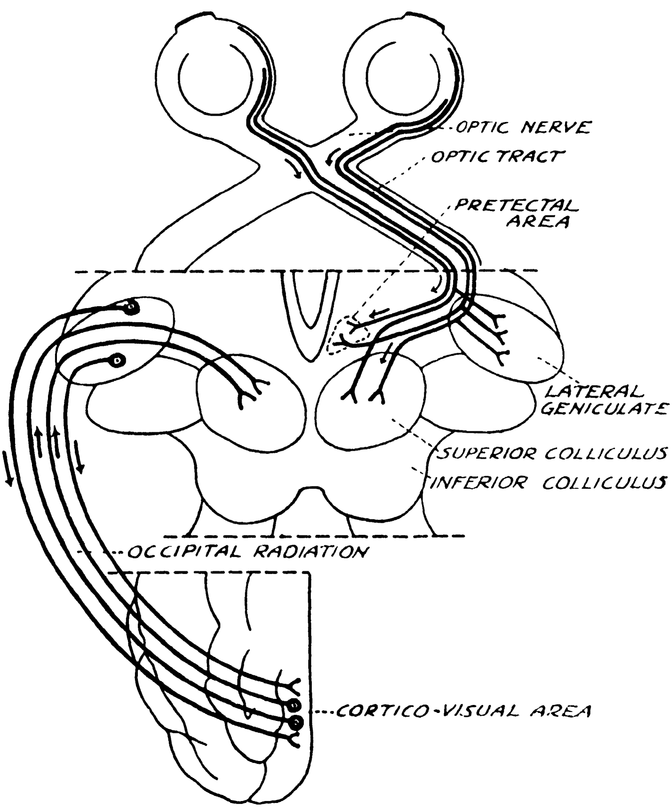

FIG. 202. Diagram of the Optic Paths For clarity the first parts of the paths are shown in the right and the final stages on the left (Modified slightly from BARRIS, *Arch. Ophthal.*, 1935, **14**, 61.)

are connected with reflex paths, the last with the transit of visual sensations to the occipital lobe.

The optic nerve contains four different sets of fibres: (1) Those which convey visual impressions to the brain; (2) those going to the reflex centres; (3) those which come down from the brain to the retinæ, the so-called 'retinomotor' fibres, which may have trophic fibres associated with them; (4) nerves travelling from one retina to the other.

(1) Owing to partial decussation at the chiasma, the visual fibres of each optic tract are derived from both eyes, viz. from the temporal half of the retina and macula of the same side, and the nasal half of retina and macula of the opposite side; thus the right optic tract contains fibres derived from the right sides of both right and left retinæ and maculæ (Fig. 202). Each optic tract connects (for vision) with the lateral geniculate body on its own side, and, after relaying there, the impulses pass onwards by the optic radiations to the visual centre in the occipital cerebral cortex. It will be seen that the occipital cortex is in this manner in connection with the halves of both retinæ of its own side. Since, as will be learnt later, each half of the retina receives light images of the opposite half of the whole visual field, this means that with vision, as with touch, each side of the brain receives impressions derived from the opposite side of the environing world.

(2) Some afferent fibres of the optic tracts pass to the superior colliculi; from this arise the tecto-bulbar and tecto-spinal tracts. This path therefore serves for reflexes which turn the head and eyes when light enters the eye.

Another reflex arc is that *viâ* the pretectal region. Probably special retinal fibres are reserved for this path, which is the pupillo-motor path. The fibres relay and then connect with the third nerve nucleus.

(3) Nothing appears to be known as to the fate of the so-called retinomotor fibres; some of them may, in fact, be trophic or vaso-constrictor fibres.

(4) The functions of the inter-retinal fibres are not known definitely. It has been suggested that they cause changes (for example, cone movement) in one retina when light falls on the other. It has also been supposed that the sympathetic inflammation which occurs in one eye after certain injuries to the other is due to impulses which have travelled *viâ* these nerves; lastly, binocular contrast and after-images have been ascribed to them.

It is of practical importance to be able to locate an injury to the visual nerve paths. Injury to an optic *nerve* causes blindness of the eye to which the nerve belongs, and exposure of the eye to light will not then elicit the pupil reflex. Injury to an optic *tract* causes blindness of the halves of *both retinæ on the same side* as the lesion, that is to say, *blindness to external objects on the opposite side* to the injury. Injury at the chiasma, *e.g.* by pituitary tumours, may only interfere with the fibres from the nasal halves of both retinæ, so causing loss of the temporal fields of vision—*bitemporal hemianopia.*

It is interesting to note that, whereas, in most other nerve paths, crossing of the fibres occurs from one side to the other as they travel to the brain, so that the left side of the brain is connected only with the right side of the body, this is not the case with the optic—or auditory—impulses. Injury to the optic radiation of the calcarine cortex will cause blindness of the corresponding halves of *both* retinæ, *i.e.* the half of each retina on the same side, but will not affect the pupil reflex, because the fibres concerned have already turned aside to go to the pretectal region.

EYE REFLEXES. *The Correlation of Head and Eye Movements.* These may be of two kinds, compensatory and associated. The compensatory ones take place when the

head is rotated in one direction and the eyes in the opposite direction, in order still to fix the gaze on some stationary object.

The associated movements occur when the head and eyes rotate in the same direction in order to fix the gaze on an object which is moving round the observer. Both these associated movements appear to be effected *viâ* the superior colliculi. These receive not only afferent fibres from the optic tracts, but also efferent ones from the occipital cortex (Fig. 202). From the superior colliculi arise the motor tecto-bulbar and tecto-spinal tracts. Probably also co-ordination is effected through the inferior olivary bodies which connect with the oculomotor nuclei *viâ* the central tegmental tract, with the upper cervical cord by the olivospinal tract, and with the cerebellum.

The Correlation of Limb and Eye Movements. For the protection of the eyes there is an important reflex which can be initiated principally by vision, but also by the other distant receptors, such as hearing. This involves (*a*) the firm closing of the lids, and, in addition, where necessary, the raising of the arm in front of the head (or the hands in front of the eyes); (*b*) the flexing of the neck and spine so that the face approaches the abdomen; (*c*) lastly, the reflex may cause the raising of the legs flexed at the knees so that they form an additional protection for the face. This reflex operates through the tecto-spinal tract.

The Pupillary Reflex. The plain muscle of the pupil is supplied by two nerves, viz. the sympathetic which causes dilatation, and the IIIrd cranial, which causes constriction. When light falls on the eye, the pupil constricts —the light reflex. This is effected by a reflex from the pretectal region, in which special fibres, or perhaps branches from the visual fibres, terminate: from here relay fibres pass to end at the IIIrd nerve nucleus, from which axons pass out to the constrictor pupillæ muscle. There is some crossing over

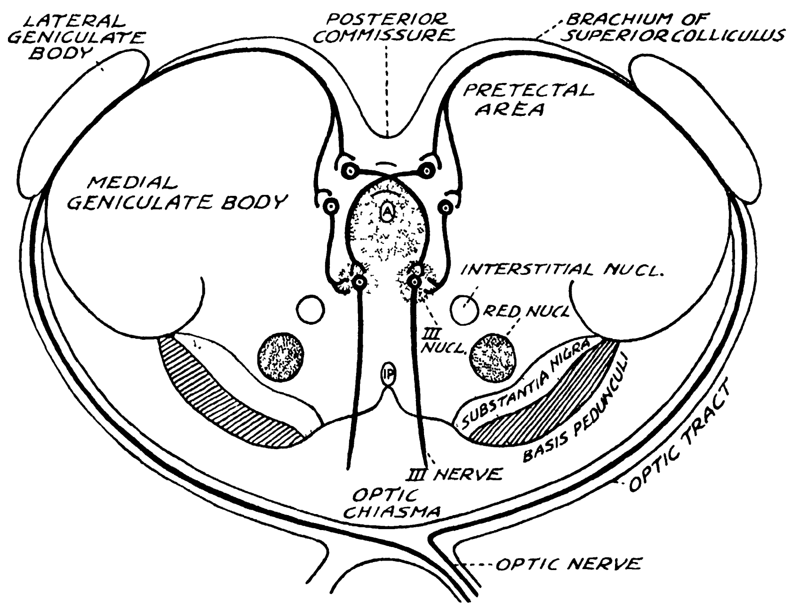

FIG. 203. Diagram of the Path Concerned in the Light Reflex. (Modified from MAGOUN and RANSON, *Arch. Ophthal.*, 1935, 13, 791.)

of neurones from side to side in the pretectal region, which explains the fact that when one eye is exposed to light the pupils of both eyes contract (Fig. 203).

In some disorders the phenomenon of the *Argyll-Robertson pupil* is encountered. In this, the pupil does not react to light, yet the IIIrd nerve

nucleus must be intact, because the pupil still contracts during accommodation of the eye for near vision. The lesion is probably in the pretectal region ; destruction of the superior colliculi, which was formerly believed to be the relay station, does not affect the light reflex,* but destruction of pretectal region as well as of the superior colliculi does.†

(4) THE PATHS FOR TASTE AND SMELL. The *nerves for taste* are the VIIth and IXth cranial, the cells of origin being in the geniculate and petrous ganglia respectively. These primary neurones end in the sensory nuclei of the VIIth and IXth nerves, from which second order fibres run, probably with the common fillet tracts of the opposite side, to the thalamus. From this, third order neurones continue to the cortical centre in the hippocampus.

The *nerves for smell* perforate the cribriform plate and enter the olfactory bulb, of complex structure, where a relay occurs. The relaying fibres form the olfactory tracts. Ultimately the impulses reach the uncinate gyrus, the hippocampus and other structures which together form the rhinencephalon or olfactory brain.

THE THALAMUS

With the sole exception of the olfactory path, all the afferent paths which are travelling to the cerebral cortex converge on to the thalamus, which is their chief relay station. Thus, it receives optic and auditory radiations, the spinal, mesial, and trigeminal lemnisci, and cerebellar fibres. It also has connections with various subcortical stations.

The thalamus has a complex anatomical structure, which is differently described by different authorities. In mammalian forms, it may be divided, according to Le Gros Clark ‡ into an upper and a lower level. *The upper level* is dorsally placed, and contains among others, the lateral and dorso-medial nuclei, the supra-geniculate and centre median nuclei. These upper levels do *not* receive direct afferent fibres, and are related to the association areas of the cortex ; they also have connections with other nuclei of the diencephalon. It is probable that the more ventrally placed ones of this group also have functions in connection with visceral control, and so connect with the hypothalamus.

The lower levels of the thalamus are chiefly ventral, and include (1) the main ventral nucleus and the medial ventral nucleus, (2) an anterior group, (3) an optic tract group, including the dorsal and ventral nuclei of the lateral geniculate body, the large-celled nucleus of the optic tract, and the pretectal nucleus, (4) the medial geniculate body.

The lower levels receive afferent impulses from the brain stem, spinal cord and hypothalamus, and relay these to the upper level nuclei and to the cerebral cortex.

Physiologically § there are three main groups of thalamic nuclei.

(i) Nuclei *with purely subcortical connections* (chiefly the anterior ventral groups). They probably serve to connect together the various nuclei of the diencephalon and have certain functions in visceral control.

(ii) Nuclei which are *relay stations on the afferent path* to the cortex. These are chiefly ventral, and include the geniculate bodies.

(iii) *Association Nuclei.* These are the dorsomedial and lateral nuclei and pulvinar. They connect with other nuclei of the diencephalon (thalamus, hypothalamus and subthalamus) and also with the parietal and anterior frontal association areas of the cerebral cortex, but receive no afferent fibres from the sensory systems.

The chief function of the thalamus is undoubtedly that of receiving, modifying and relaying sensory impressions to the cortex.

Removal of parts of the cerebral cortex leads to degeneration of the thalamic relay nuclei connected with those parts. Thus it can be shown that

* KELLER and STEWART. *Amer. J. Physiol.*, 1932, **101**, 64.

† MAGOUN. *Amer J. Physiol.*, 1935, **111**, 91 , RANSON and MAGOUN. *Arch. Neurol. Psych.*, 1933, **30**, 1193 ; MAGOUN and RANSON. *Arch. Ophth. N.Y.*, 1935, **13**, 791, 862 ; BARRIS. *Arch. Ophth. N.Y.*, 1935, **14**, 61.

‡ LE GROS CLARK. *Brain*, 1932, **55**, 406.

§ EARL WALKER. *J. Nerv. Ment. Dis.*, 1937, **85**, 249.

the lateroventral parts project to areas 4 and 6 (*v.* p. 316) of the cortex and are concerned with unconscious kinæsthetic afferents, while the posteroventral parts act as relay stations for conscious sensations arriving *viâ* the lemnisci on their way to the cortex. Hence destruction of the posteroventral portion of the thalamus causes disturbances of skin and kinæsthetic sensations on the opposite side. The representation of areas is fairly precise in the thalamus.

The lateral geniculates, as we have already seen, are relay stations on the visual path, and the medial geniculates on the auditory and vestibular paths. The pulvinar serves to correlate visual and auditory impressions

Disease of the thalamus is often accompanied by extreme hypersensitiveness in the areas represented. This is probably a release phenomenon due to severing of cortical connections. There seems little doubt that the thalamus is capable of sensation of a crude type independently of the cortex. This explains why some responses, especially to pain and temperature, may remain even after the removal of large areas of the cerebral cortex.

We have already seen that the decorticate animal exhibits a tendency to a display of 'sham rage' on receipt of almost any stimulus, and this is believed to be due to the removal of the normal control of the cortex by which such emotional expressions are toned down. It is probably in the upper levels of the thalamus that afferent impressions become loaded with an emotional quality.

II. UNCONSCIOUS AFFERENTS

The paths used for conscious impressions may also often be traversed by impulses that do not emerge into consciousness, but are concerned wholly with reflex effects Some paths, however, are devoted wholly to the transmission of impulses which normally arouse no sensations If, by exceptionally strong stimulation, such impressions do force their way into the field of consciousness, the sensations aroused are vague in character and lacking in local sign. The most important of these tracts are concerned with unconscious kinæsthetic impressions, related to the position and movements of the limbs, neck or trunk Their most important destination is the cerebellum Others lead from the viscera, and have connections with the spinal and bulbar centres Among these paths the following are important —

(1) *The spinocerebellar tracts* arise from the secondary neurones of the Clarke-Stilling column in the spinal cord. The axons of these cells pass into the *dorsal and ventral spinocerebellar tracts*, chiefly of the same side and ascend through the medulla. Here the dorsal spinocerebellar tract takes a more dorsal position and forms an important part of the restiform body, or inferior cerebellar peduncle, with which it travels to, and ends in, the cortex of the vermis of the cerebellum.

Other important afferents originating from the dorsal columns of the cord reach the cerebellum from the bulbar nuclei, chiefly of the opposite side, *viâ* the restiform body. Among these are axons (the arcuate fibres) from the nuclei Gracilis and Cuneatus, which relay unconscious kinæsthetic impulses ascending the spinal cord.

(2) *Vestibular Paths.* The fibres of the vestibular nerve form synapses with cells in one of the four subdivisions of the vestibular nucleus, viz. medial (Schwalbe), lateral (Deiters), superior (Bechterew), or the vestibulo-spinal nucleus. From the cells of these nuclei four main tracts arise :—

(i) *Connects with the posterior longitudinal* bundle of both sides.

(ii) The *vestibulo-spinal tract*, which descends homolaterally in the cord.

(iii) *The vestibulo-reticular tract,* which connects with motor nuclei and reticular formation, of opposite side.

(iv) *The vestibulo-cerebellar tract,* which includes some direct fibres from the vestibular nerve. It is homolateral.

In addition to these tracts leaving the vestibular nucleus, there is the cerebello-vestibular or uncinate facsiculus, which descends to the vestibular nucleus from the roof nuclei of the cerebellum of the same side.

(3) *Olivo-cerebellar Tracts.* The olivary bodies have afferent connections with the spinal cord, thalamus, etc., and send out fibres which pass up in the inferior peduncles to the cerebellum.

(4) *Visceral Afferents.* These will be considered later.

CHAPTER XX

THE CEREBRAL HEMISPHERES AND THE EFFERENT PATHS

THE cerebral hemispheres form the largest and most important part of the human brain. It is to the progressive evolution of this part that is due the rise in type in vertebrates. In development they are formed as two diverticula from the front part of an outgrowth of the first cerebral vesicle. In the lowest vertebrates these outgrowths are connected entirely with the olfactory sense organs, and we may regard the olfactory brain as a fundamental structure on which has been built up all the rest of the cerebral hemispheres. With the increasing importance of the visual sensations in the bony fishes there is still very little corresponding growth of the fore-brain, most of the fibres from the optic nerves going to the roof of the mid-brain (the optic lobes). The beginning of the cerebral hemispheres is associated with the development of nervous tissue in the roof of the fore-brain. At its first appearance this higher brain material still receives chiefly olfactory impressions. But the structure of the cerebral cortex thus laid down differs from that of the centres forming the brain stem or the olfactory lobe itself in that it provides for a very rich association of impulses between all its parts. The fibres entering the cortex break up into a fine meshwork of fibres which run tangentially to the surface and come in relation with innumerable dendrites of nerve cells situated at some little distance below the surface. We have here the first germ of an apparatus in which the nerve paths can be determined by education. In the amphibian and reptile, the cerebral cortex extends over the whole roof of the cerebral hemispheres, though even here a very large proportion of it is devoted to the association of olfactory impulses. The wider reactive powers of birds are based chiefly on an enormous development of the corpus striatum, whereas in mammals the chief development occurs in the roof of the cerebral hemispheres. With the increased entry of fibres, carrying impulses from all the other sense organs of the body, the olfactory part of the brain diminishes in importance, and in the higher mammals and man is altogether overshadowed by the newly formed structures of the cerebrum. The cortex of the olfactory brain is sometimes called the *allocortex*, and that of the remainder of the cerebrum the *isocortex*.

The whole surface of the brain is thrown by fissures into convolutions, by which means about a threefold increase of the surface grey matter is obtained. By the larger fissures the brain surface is divided into lobes. The chief lobes are (*a*) the frontal ; (*b*) the parietal ; (*c*) the occipital ; (*d*) the temporal ; (*e*) the limbic area. The chief fissures separating these are (*a*) the central or fissure of Rolando, between frontal and parietal lobes ; (*b*) the Sylvian fissure between the parietal and temporal ; (*c*) the parieto-occipital ; (*d*) the calloso-marginal, forming the outer boundary of the limbic area. Each of the main lobes or areas mentioned above is further subdivided by numerous fissures.

MINUTE STRUCTURE OF THE CEREBRAL CORTEX. The cortex of the cerebral hemispheres consists of a layer of grey matter covering a central mass of white fibres. The white fibres are axons passing out of or into the grey matter. Examination shows that the naked eye appearance of the grey matter, in section, differs from place to place.

Histologically the grey matter consists of layers of nerve cells embedded in neuroglia and nerve fibres, both medullated and non-medullated. The nerve cells vary in size and shape ; one cell typical of the cerebral cortex is the *pyramidal cell*, a cone-shaped cell with one large apical process which runs towards the surface, various dendrites which are given off from the sides and lower angles of the cell, and another process (usually the axon) from the base

of the cell which passes downwards giving off small collaterals in its course. Some of their axons (*projection fibres*), including those which form the pyramidal tracts, pass by the corona radiata through the internal capsule and into the crura cerebri. Others, or their collaterals, may pass into the adjacent regions of the cortex, or by the corpus callosum into the opposite cerebral hemisphere (*association fibres*). Other neurones belong to Golgi's type II and are confined to the grey matter ; they are called "*intracortical neurones.*"

The structure of the allocortex (olfactory) * is totally different from that of the isocortex and cannot be considered here.

In the isocortex, histological examination of the layers of cells and fibres shows that there are many different variations of a presumably common plan of stratification, and much labour has been devoted to an attempt to unfold this basic plan. When the whole cortex of man is systematically studied it is found that it can be divided into numerous areas each of which has a distinctive structure, as judged by the thickness of the various layers of fibres and cells and by special modifications which they show from one area to another. The number of fields into which the whole cortex is thus divisible varies according to the criteria adopted by different investigators, and has been increased as a result of detailed study from an initial twenty areas † to fifty, ‡ or even, according to some, to more than 200, all of which are presumed to have different functions.

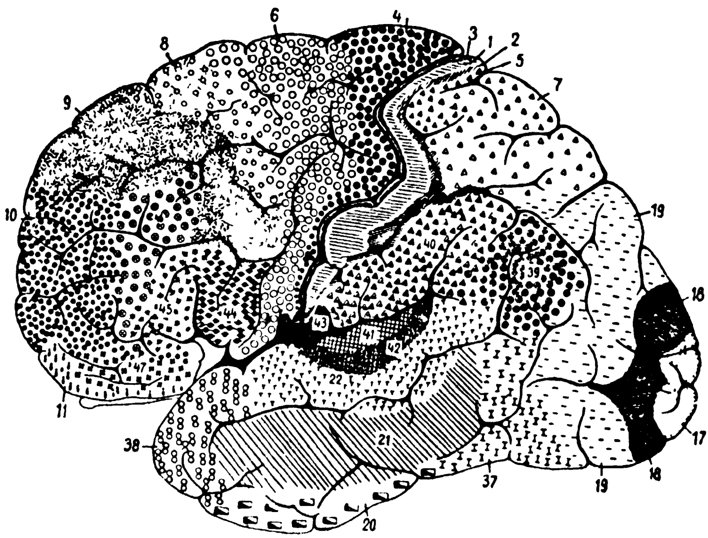

FIG 204 Brodman's Cytoarchitectonic Map of the Human Cortex (From FULTON's "Physiology of the Nervous System," Oxford Univ. Press.)

Some of the more generally accepted structurally distinguishable areas are shown in Fig. 204, and it is common to refer to the areas by the numbers there given.

* LORENTE DE NÓ. *J Psychol Neurol.*, 1934, **45**, 381 , **46**, 113

† CAMPBELL. "Histological Studies on the Localisation of Cerebral Function," Cambridge University Press, 1905.

‡ ECONOMO. "The Cytoarchitectonics of the Human Cerebral Cortex." London, Oxford Univ. Press, 1929 ; BRODMANN, *Vergleichende Lokalisationslehre der Grosshirnrinde*, etc. Leipzig, Borth., 1909 and 1925.

The cell layers described are recognised by staining the cells for Nissl's granules.

The plan of structure of the important parts, represented by the parietal, temporal and occipital lobes, generally agreed upon is that of a fundamentally six-layered stratification of cells of different types, layers IV., V. and VI., however, being often subdivided. If the medullated fibres are stained, as by the Weigert-Pal method, instead of the cells, new strata, consisting of fibres, are revealed (Fig. 205).

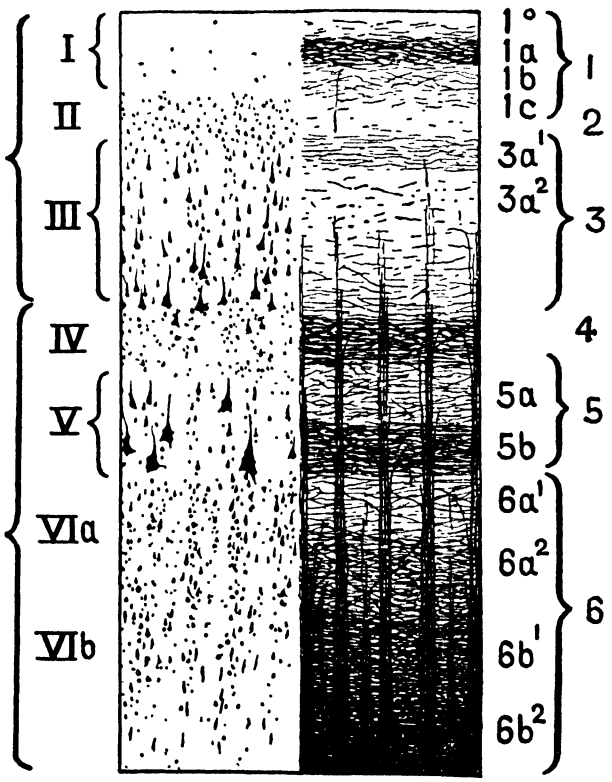

FIG. 205. Diagrammatic Representation of the Layers of the Human Cerebral Cortex according to Brodmann and Vogt. On the left the six cell layers. On the right the fibre layers, Layers 4 and 5b being respectively the outer and inner lines of Baillarger. (S. WRIGHT, after ECONOMO, 'Cytoarchitectonics of the Human Cerebral Cortex,' Oxford Univ. Press, 1929.)

These six cell layers, in two laminæ, are :—

External Lamina

I. The plexiform layer. It contains few cells, and is composed principally of the dendritic endings of cells belonging to deeper layers. It contains a few horizontal cells of Cajal, with processes which run approximately parallel to the surface.

II. The layer of small pyramidal and small granule cells.

III. The outer layer of medium pyramidal cells.

IV. (*a*) The layer of star pyramids. (The outer line of Baillarger (fibres) is at this level.)

IV. (*b*) The layer of stellate cells. Sometimes this layer is called the middle stellate or granule layer.

Internal Lamina

V. The inner layer of large deep pyramids. It is in this layer of the motor area that the giant Betz cells are found. (The fibre component of this layer, 5*b*, is the inner line of Baillarger.)

VI. The fusiform cell layer

Internal to layer VI is the white matter of the cerebral cortex.

The pyramidal cells of all sizes possess, in general, three different kinds of fibre: an apical one, which runs towards the external surface, and then branches tangentially to the surface; a basal one (axon) running towards or into the white matter, where it becomes medullated and runs a variable distance according to its function; and a series of transverse collaterals, which run tangentially. In consequence, these tangential fibres form well-marked lines, particularly at layers IV and V. In consequence, if sections are stained by a method like Weigert's, sheaves of radial fibres will be seen running from the white matter towards the surface, giving off a rich meshwork of fibres to the intervening portions of the grey matter, while the bands of tangential fibres are seen running parallel to the surface. Thus we find (Fig. 205): (1) The tangential fibre layer which corresponds to the plexiform layer; (2) a layer of tangential fibres at layer IV, corresponding to the stellate cell layer. This is sometimes called the outer line of Baillarger; (3) an inner line of tangential fibres at layer V, corresponding to the layer of large deep pyramidal cells, sometimes called the inner line of Baillarger.

Two of the most clearly marked differences in areas are: (1) In area 4, the motor area, layer V is occupied by giant Betz cells, the axons of which form

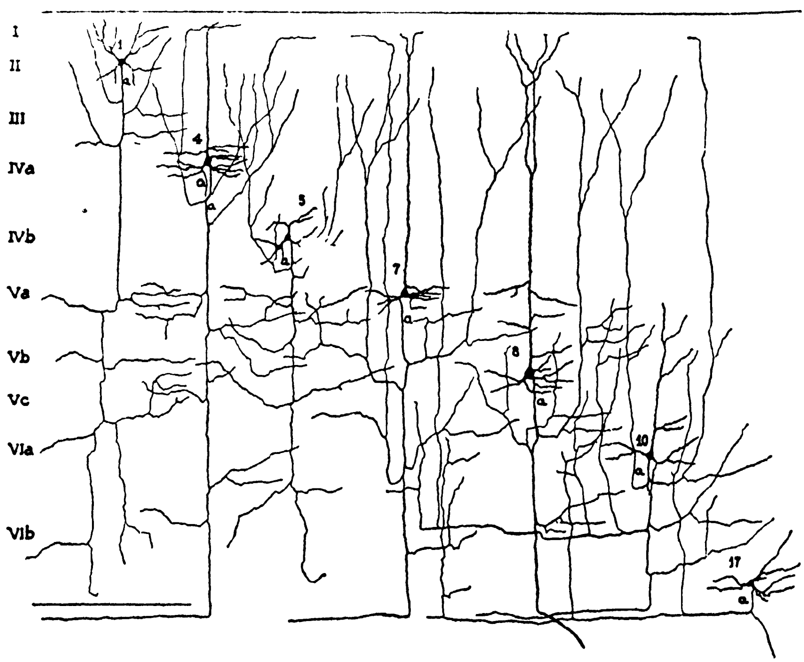

FIG. 206. Form and Position of Descending-axon Neurones of Cerebral Cortex. Axons marked *a*. Note that collateral branches are concentrated in layers I–III and V–VI. Axons of cells 1 and 5 are confined to the cortex; those of 4, 7, 10 and 17 are association fibres; that of 8 is a projection fibre. (LORENTE DE NÓ, in FULTON'S "Physiology of the Nervous System," Oxford University Press, 1938.)

the pyramidal tract. (2) In area 17, the visual area, the pyramidal cell layers are thin, the large pyramidal cells being very few. The lines of Baillarger are very distinct, particularly the outer, which is sometimes called the line of Gennari, a characteristic of this region.

Although this mapping of the cortex is valuable in stressing the varying structure of its different parts, it tells us little with regard to the functional connections of the neurones. For this purpose sections stained by Golgi's method are needed, and Figs. 206, 207 and 208 illustrate the findings thus made. It can then be realized that, as stressed by Lorente de Nó, the important point, as regards functions, is not so much the horizontal lamination as the fact that the cells are arranged in vertical chains, with synaptic connections at definite levels characteristic of each type of neurone. Seen from this standpoint we can recognize four main types of neurone in the cortex :—

(1) Cells with *axons that descend*, often into the white matter, where they form projection or association fibres (Fig. 206 1 to 10, and Fig. 208).
(2) Cells with *axons that ascend* and ramify in other layers (Fig. 207, 18 to 21).
(3) Cells of Golgi type II, with short branching axons (Fig. 207, 23 to 26).
(4) Cells with horizontal axons (Fig. 207, 22).

It will be noticed that, with the exception of the projection and association neurones of the first group, all these neurones are intra-cortical, *i.e.* they must have internuncial functions.

Some typical neurones are shown in their proper positions in the layers, in Fig. 206 (descending axons), Fig. 207 (intra-cortical neurones, some with ascending axons) and Fig. 208 (general view of all types, and of the typical afferent fibres that reach the cortex).

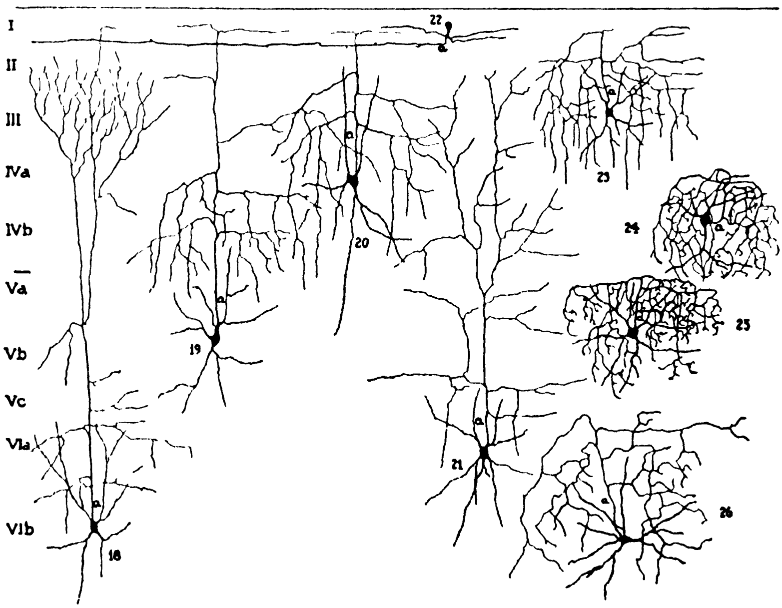

FIG. 207. Types of Cerebral Cells with Intracortical Axons: axons marked *a*: 18 to 21, cells with ascending axons; 22, cell with horizontal axon; 23 to 26, cells with short axons. (LORENTE DE NÓ in FULTON'S "Physiology of the Nervous System," Oxford University Press, 1938.

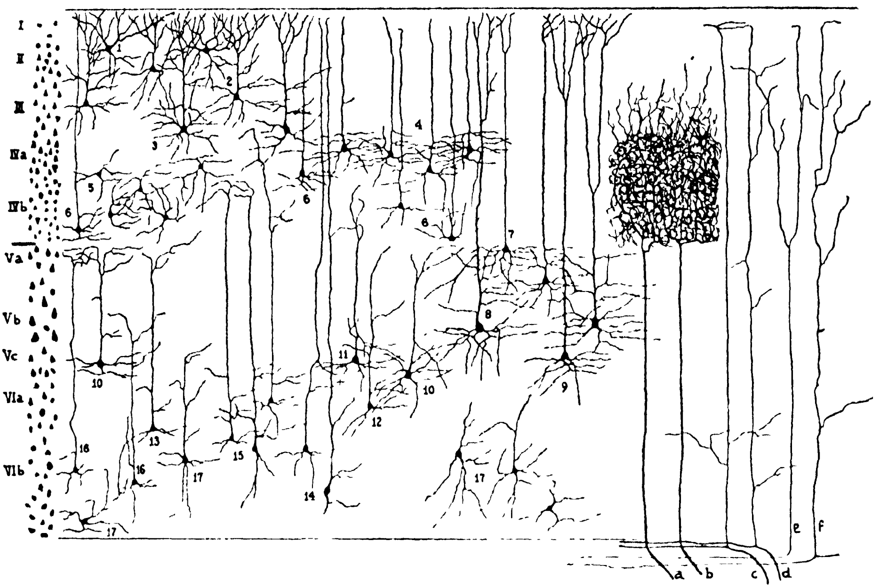

FIG. 208. Parietal Cortex of Mouse On left a strip showing the cell layers On right the principal afferent fibres, of which *a*–*d* are from thalamus, and *e*, *f* are association fibres Main picture shows the relation of the chief types of descending axon cells to the cell layers, and the position of their dendritic branches. *For clarity of drawing the descending axons have been omitted*, but are shown in Fig. 206.

The cells are . 1, Pyramids of layer II
2, 3, Pyramids of layer III.
4 to 6, Star cells and pyramids of layer IV.
7 to 11, Pyramids of layer V.
12, 13, Pyramids } of layer VI.
14 to 16, Spindles } of layer VI.
17, Star cells } of layer VI.

(LORENTE DE NÓ, in FULTON'S Physiology of the Nervous System," Oxford University Press, 1938)

In its general plan, as emphasized by Lorente de Nó,* the cerebral cortex appears to consist of chains of neurones, arranged in a generally vertical direction. Afferent fibres, from the thalamus or from other parts of the cortex, pass vertically upwards, and give off collaterals to all cell levels, except layers I and II. These collaterals form synaptic connections with all the types of cell, and we may speak of the afferent fibre, with all its connections as an elementary cortical unit. In this unit the afferent impulse passes over to the efferent fibre. The general connections within an elementary unit, according to Lorento de Nó, are shown diagrammatically in Fig. 209.

The efferent fibres which emerge may pass, as association fibres, to other parts of the cortex, or as projection fibres to more distant parts.

A careful study of Fig. 209 and of the simplified diagram to the right of it, shows that a nervous impulse, entering the unit by an afferent fibre, makes synaptic contact not only with an efferent neurone, but also with numbers of internuncial neurones, by which reverberatory and delaying circuits are formed as by-passes, so that impulses are delivered over and over

* LORENTE DE NÓ. Article on Structure of Cerebral Cortex, in FULTON'S "Physiology of the Nervous System," Oxford University Press, 1938

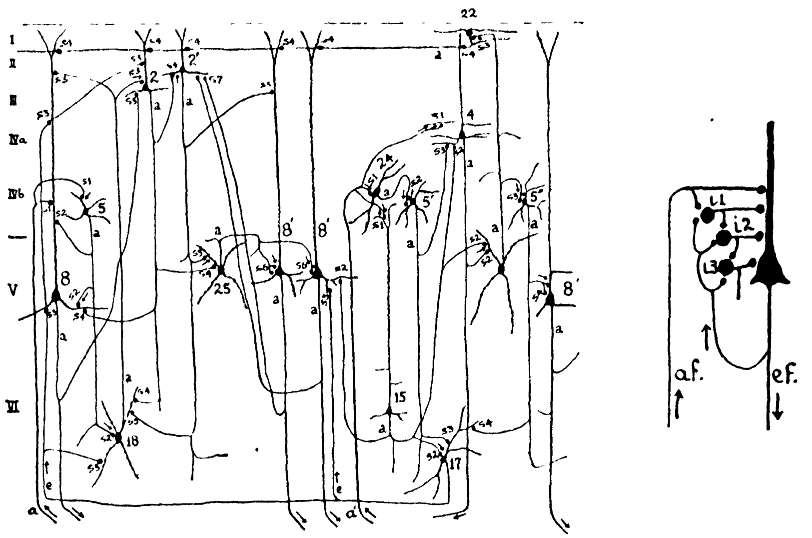

FIG. 209. Diagram of some of the Intracortical Chains of Neurones The numbers on the cells, and the letters a and e on the fibres, are the same as in Figs 206, 207 and 208 The axons are marked with a Only a few dendrites and axonal branches have been included in the diagram The synaptic junctions are indicated with the letter s (s_1, s_2, etc) and with a thickening of the axon. It is assumed that the synapses marked with an arrow are passed by the impulses.

The small diagram at the right is a simplification of the diagram at the left The afferent fibre *af* activates the large pyramid, which is the origin of an efferent fibre *ef*, and also a system of cortical internuncial cells (i_1, i_2, i_3), the recurrent collateral of *ef* delivers impulses again to the internuncial system This diagram summarizes the plan upon which the cerebral cortex is built (LORENTE DE NÓ, in FULTON'S "Physiology of the Central Nervous System," Oxford University Press, 1938)

again to most of the cells, in the manner previously outlined. These trains of impulses not only result in more prolonged bombardment of the neurones, but, as a result of facilitation, may open up synapses not previously permeable.

So an entering impulse may, for instance, pass first to layer IV, thence down to layers V and VI and again up to layers II and III, finally emerging from a layer characteristic for the particular area.

It should be noted that all these arcs are superposed on the direct one from afferent to efferent, as *e.g.* from fibre a to cell 8 in Fig. 209. Further, there are various possible closed-chain circuits, as *e.g.* 8-2, 18-8, etc.; in the figure a, a' are afferent fibres, 8, 8′ and 8″ are effector neurones; the others (including 4 and 17, which give association axons e) are internuncial neurones. Some of the by-path arcs are confined to one layer, *e.g.* 25-8′, while others are long and span several different layers, *e.g.* 18-2. In the higher animals there are proportionally more of the short-axon connections, which confer greater delicacy and range of paths.

LOCALIZATION OF FUNCTION IN THE CORTEX

It has been found by experimental removal or stimulation of parts of the cortex that different areas of the cerebral cortex are closely associated with certain functions. Some areas are the site of origin of efferent projection fibres concerned with movements—the *motor areas*, while others, the *sensory areas*, are concerned with the reception and appreciation of sensory impulses. The motor projections fall into two groups: (1) The pyramidal, the axons forming the pyramidal tracts; (2) the extra-pyramidal, which emerge by

other routes. The relation of the chief of these areas to the areas on the structural map (Fig. 210, but not all areas are shown) are briefly as follows :

THE FRONTAL LOBE. Area 4, the Betz cell area, is *motor*. In man this area does not occupy the whole of the precentral gyrus, but only the anterior lip of the Rolandic fissure. The axons of the Betz cells form the pyramidal tract, and pass down to the motoneurone pools in the brain stem and spinal cord.

Area 6, the so-called '*pre-motor*' *area*, occupies the greater part of the precentral gyrus. It has connections with area 4 and also sends some fibres into the pyramidal tract. It also has motor projections, but these are often supposed to be largely concerned with the extra-pyramidal system, and with the musculature of the mouth and respiration. Its lower parts (44 and 45) sometimes called Broca's area, are concerned with speech.

Area 8 is also largely extra-pyramidal and concerned with *eye movements*.

Areas 9 *to* 12 is the prefrontal area or frontal association area, and is probably *associative* in function.

THE PARIETAL LOBE. Areas 3, 1, 2, the 'post-central' area, is sensory, receiving most of the projections from the thalamus, for *cutaneous and general kinæsthetic sensitivity*.

Areas 5 *and* 7 are also mainly sensory.

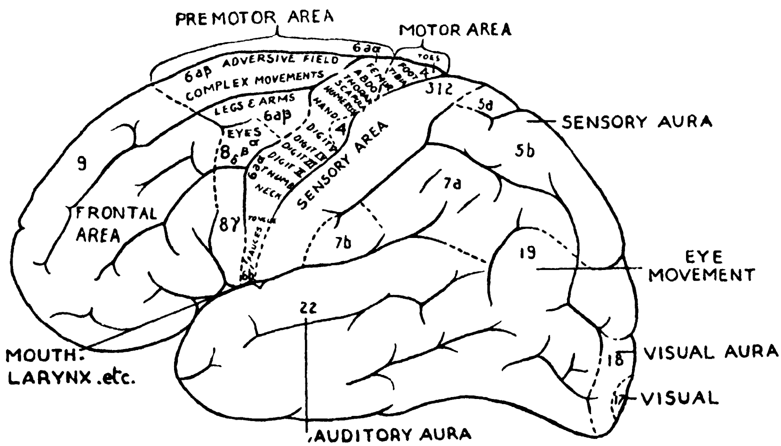

FIG. 210. Diagram of the Chief Cortical Areas in Man, according to FOERSTER and PENFIELD. (Slightly modified from *Ztschr. f. d. ges. Neurol u. Psych.*, 1930, **125**, 475.)

THE TEMPORAL LOBE, Areas 41–42, receive the *auditory* radiations and are sensory for hearing.

Area 22 is an *auditory association* area.

THE OCCIPITAL LOBE is entirely visual in function.

Area 17, the true *visuo-sensory* or 'striate' area, receives projections from the lateral geniculate body and also sends fibres to it.

Area 18 is *visual associative*.

Area 19 is a visual area connected chiefly with the thalamus. Stimulation causes eye movements as a secondary effect.

ASSOCIATIVE FUNCTIONS OF THE CORTEX. The earlier investigations on localization led to a prevalent belief that it would ultimately be possible to allocate a precise function to every part of the cortex, in such a way that we should be enabled to forecast a particular loss of function when a particular portion of cortex was damaged, or a precise effect on stimulation. This has proved not to be the case, and it must be admitted that large areas of the cortex do not possess precisely delimited functions,

but rather exercise general functions of some kind. Thus, large parts of the frontal lobes (areas 9 to 12) are quite unresponsive to electrical stimulation, and these areas on one side (notably on the right side) may be removed surgically without very evident effect. Removal of the greater part of the areas 9 to 12 on both sides does, however, lead to general mental impairment.* It seems, therefore, that these portions of the frontal lobes possess associative functions which are, however, probably shared by other areas of the cortex, so that they cannot, so to speak, be regarded as the seat of the 'organ of mind.'

It is otherwise with the places, the sensory centres, at which sensory impressions are received, and with those, the motor areas, from which efferent impulses leave the cortex. The receiving and emitting apparatus do have reasonably fixed positions whereas the association areas deal more or less as a whole with the complex processes that are interpolated between receipt of an impression and the consequent motor response.

SENSORY AREAS IN THE CORTEX

The different types of afferent fibres that reach the cortex travel to definite areas according to their functions. The chief of these areas are in the parietal, temporal and occipital lobes.

To begin with general body sensations. These we have traced up from their origin on the opposite side of the body *viâ* the spinal, mesial and trigeminal lemnisci to the thalamus, at which level there appears to be a crude recognition of the particular quality of the sensation, coloured by an affective quality (*i.e.* a definitely painful or pleasurable property). Impulses are relayed on from the thalamus to the cortex of the parietal lobe, which they reach by passing through the internal capsule and corona radiata. The area concerned is sometimes called

THE SOMÆSTHETIC AREA. Its chief part includes the posterior lip of the fissure of Rolando and the greater part of the post-central gyrus lying posterior to it (areas 1 to 3). This post-central area has often been subjected to direct electrical stimulation in human subjects † with the result that sensations of numbness, pressure, touch or warmth were felt on the opposite side of the body, the actual place depending on the part of cortex stimulated. Thus, stimulation of the upper part of the area gave sensations in legs or feet; of the middle part, sensations in trunk or arm, and of the lower part, sensations in face and head. There was no special localization of different types of sensation, but only representation of places stimulated.

According to some, sensation is also caused by stimulation of the corresponding parts of the anterior lip of the Rolandic fissure,‡ although, as we shall see later, this area is also definitely motor. Powerful excitation of the posterior parietal areas 5 and 7 may also produce sensation.

Another method of studying these areas in animals is by the local application of strychnine to the cortex.§ This causes an excitatory condition to spread out over the cortex from the place of application, and the animal responds by paying attention, *e.g.* by scratching, to the part of the body represented. The results are generally in agreement with those obtained on man. Yet another method is to record the potential changes on the surface

* JEFFERSON. *B. Med. J.*, 1937, (ii) 199.
† CUSHING. *Brain*, 1909, **32**, 44.
‡ PENFIELD and BOLDREY. *Brain*, 1937, **60**, 389.
§ DUSSER DE BARENNE. *Q. J. Exp. Physiol.*, 1916, **9**, 355; *Proc. R. S.*, 1924, **96B**, 272.

of the brain when the skin is stimulated by any mechanical means, and to note from which regions characteristic waves can be recorded.

Ablation of the areas concerned, or of parts of them, leads to loss of the finer grades of discrimination in sensation in the part represented * ; but not to any complete loss of sensation. There is also clumsiness and inaccuracy in the movements of the parts concerned, and there is evidence that some of the cutaneous part of the sensations is bilaterally represented.

According to Head and Holmes the chief function of the somæsthetic area is the integration and critical examination of the crude sensory phenomena that are submitted from the thalamus. It thus has discriminative functions enabling us to form opinions regarding (1) relative *intensities* of sensations, *e.g.* of warmth; (2) *spatial relationships, e.g.* the position of limbs, the exact locality of a skin stimulus; (3) *similarities and differences* such as are arrived at by a synthesis of all the sensations involved. Thus we realize by touch similarities (or differences) of size, weight, texture. This is sometimes called *stereognosis*.

For Reference

BARD (1938). Studies on the Cortical Representation of Somatic Sensibility. *Harvey Lects.*, 1937-38, p. 143.

THE AUDITORY CENTRE. We have traced the fibres from the auditory nerve as far as the *medial geniculate body*. From this a new relay starts and, passing *viâ* the posterior limb of the internal capsule forms the *auditory radiation*, which ends in the auditory cortex in areas 41, 42. Not only is this sense served by the part of the cortex visible on the external surface of the temporal lobe, but also by cortex concealed from view (the gyrus of Heschl) in the depths of the Sylvian fissure. The cortex is divided physiologically into two parts, the auditory-sensory area, and the audito-psychic area.†

The *audito-sensory* area (41 and 42) is largely hidden in the Sylvian fissure. A small part, however, is seen at the superior lip of the temporal lobe. Here the stimuli from the cochlea are interpreted as sensations of sound which vary in pitch, loudness and quality. The audito-sensory area is marked histologically by all its layers being very rich in both cells and fibres, particularly is this the case with the outer layer of large pyramidal cells, which are both large and numerous. So also are the stellate cells in the layer just internal to this. These features are typical of the audito-sensory area. When sections are stained to show nerve fibres they exhibit a well-marked band running parallel to the external surface, *the line of Kaes*. The vestibular nerve has more important relations to the cerebellum, but it also has cortical connections with the temporal auditory area.

Electrical stimulation of the auditory area of one side in conscious human subjects gives rise to subjective noises in both ears, and, because both sides are represented, a temporal lesion on one side, though it may cause some impairment of hearing, does not cause deafness.

The more peripheral portions of the auditory area in the lower two temporal gyri (area 22) form the *auditory-psychic* area. Histologically, the cells found are small and there is no line of Kaes. This is an associative centre which is concerned chiefly with the appreciation of the significance of the sounds. Stimulation causes auditory hallucinations and movements of the head to the opposite side. A special part of the superior and middle

* HEAD and HOLMES. *Brain*, 1911, **34**, 102.

† BREMER and DOW. *J. Neurophys.*, 1939, **2**, 308.

temporal convolutions on the left side (in right-handed people) is concerned with the understanding of speech, since failure of speech, or to understand spoken words, results from lesions in that situation.

THE VISUAL CENTRE. The cortical centre for vision is the 'area striata' situated on the walls of the calcarine fissure (area 17). The visuo-sensory area on section shows to the unaided eye a conspicuous white striation known as the *line of Gennari*, which lies roughly midway between the two boundaries of the grey matter. It is formed by the interlacing dendrites of large stellate cells which replace the external layer of large pyramidal cells found in other parts of the cerebral cortex. This layer of large stellate cells is broad and is the most conspicuous feature of the visuo-sensory cortex. Other noteworthy features are the solitary giant pyramidal cells of Meynert, which are found forming a row of widely separated cells in the internal layer of large pyramidal cells.

To this area the fibres from the lateral geniculate body pass *viâ* the posterior limb of the internal capsule and the optic radiations (Fig. 202, p. 303). The visual cortex on each side receives impulses from its own side of both retinæ, so that it is concerned, like most other cortical centres, with impressions arising from the opposite side of the surrounding world. There is fairly accurate spatial representation of retinal surfaces on the visual cortex. Thus the upper quadrants of the peripheral retinæ are connected with the parts of the cortex above the calcarine fissure and the lower quadrants with parts below it. The macula is separately represented near the posterior occipital poles.

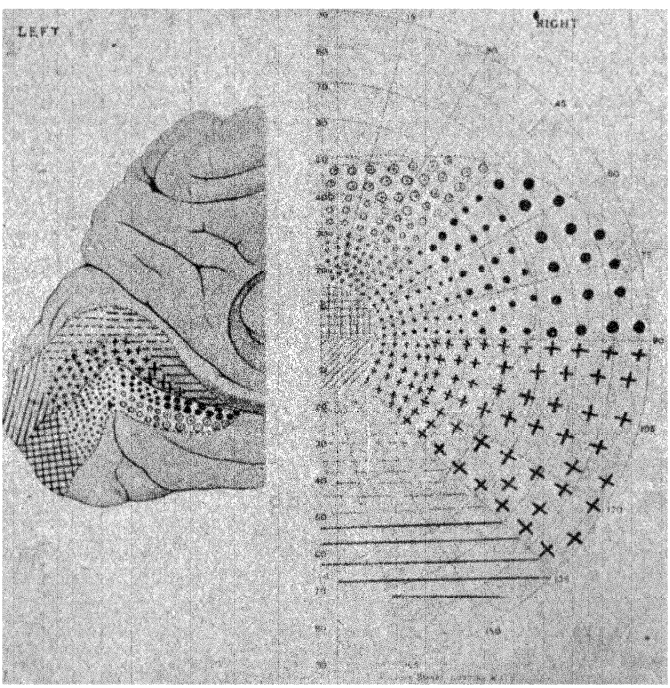

FIG. 211. The Relationship between the Field of Vision and the Calcarine Cortex. (After GORDON HOLMES.*)

* HOLMES and LISTER *B Med J*, 1919, ii, 193.

Visual sensation is located in area 17, which is sometimes called the visual sensory area. Sensations of light and movements of the eyes result from stimulation of almost any part of the cortex surrounding the calcarine fissure. If the upper surface of the right calcarine zone be stimulated, both eyes move downwards and towards the left. Excitation of the lower part causes movement of the eyes up and to the left; while between these two parts there is an intermediate zone, most marked on the mesial surface, stimulation of which evokes a purely lateral deviation of the eyes to the left. Destruction of this area on one side causes *homonymous hemianopia, e.g.* if the right visual area is ablated there is loss of function of the right half of both retinæ which means blindness in the left half of the visual fields of both eyes. The location of the visual centre in the area striata is further proved by the fact that cortical electric potentials aroused by light on the eye can be traced to this area.*

The adjacent areas 18 and 19 and part of the posterior parietal area are concerned with the association of visual and other impulses. Here visual and tactile, and to a lesser extent auditory impressions are correlated and synthesized to give proper visual perception and appreciation. A person born blind and having sight restored when adult is unable to understand the meaning of visual sensations until by a process of re-education these have been compared with the familiar tactile and auditory ones. Lesions in areas 18 and 19 in man result in disturbances of visual appreciation, so that written or printed words may lose their significance. Stimulation of area 19 causes visual hallucinations, and movements of both eyes to the opposite side.

SMELL AND TASTE CENTRES. These are situated in the uncus and the anterior part of the hippocampal gyrus near to the auditory centre. Hence lesions in the temporal lobe often result in conjoint deficiency in hearing, taste and smell.

MOTOR AREAS OF THE CORTEX

There are several areas of the cortex, the stimulation of which results in precise muscular movements, and such areas are called *motor areas*. The most important ones are in areas 4 and 6, which are motor areas proper. Area 4 in man occupies the anterior lip of the fissure of Rolando, and area 6 most of the remainder of the precentral convolution. The areas extend on to the mesial surface of the hemisphere. Movements of eyes can also be obtained by stimulation of area 8 of the frontal field, and of area 19 of the occipital lobe, while turning of head, eyes and trunk to the opposite side is obtained from the temporal lobe in area 22 and from a parietal field in area 5.

The earlier stimulation experiments were made on the lower mammals, in which the anatomical relations are somewhat different and need not be considered here, but valuable information was obtained from them and extended and applied in experiments on monkeys and the apes.† Ultimately many experiments have been carried out on the human subject,‡ and these have confirmed the inferences drawn from the work done on the apes. In the dog the motor areas are small and more diffuse, but in man the localization is well defined, especially in area 4.

* KORNMÜLLER. *Dtsch. Z. f. Nervenheilk.*, 1933, **130**, 44

† GRUNBAUM and SHERRINGTON. *Proc. R. S.*, 1901, **69**B, 206; 1903, **72**B, 152; LEYTON and SHERRINGTON. *Q. J. Exp. Physiol.*, 1917, **11**, 135.

‡ BIDWELL and SHERRINGTON. *Brit. Med. J.*, 1893, (ii) 988; PENFIELD and BOLDREY. *Brain*, 1937, **60**, 389.

STIMULATION OF MOTOR CENTRES. If any part of the precentral convolution be stimulated with a weak faradic current, movements are produced on the *opposite* half of the body, the site of the movements depending on the locality of the spot stimulated.

It was formerly a subject of dispute whether the movements resulting from stimulation of the cortex were due to the excitation of the grey matter or of the underlying white matter. The following facts show that the seat of the excitation is in the grey matter :—

(1) A smaller strength of current is required to excite the grey matter than the underlying white matter, after removal of the grey matter.

(2) Inexcitability of the grey matter can be produced by painting it with cocaine.

(3) The latent period elapsing between the stimulation and the occurrence of the movement in the corresponding limb is longer when the grey matter is excited than when the stimulus is applied to the white matter.

Whether the stimulus acts directly on the pyramidal cells of the cortex, or whether, as seems more likely, it is the endings of the afferent nerves to the cortex which are really excited by the stimulus, we cannot at present determine.

Sub-threshold stimuli may be facilitated and so rendered adequate by preceding weak stimuli.*

We may say broadly that, by stimulations of the precentral convolution at a series of points from above downwards, we get movements in that order of the leg, arm and face ; though, as is shown in the diagram (Fig. 210), within these larger areas smaller areas can be distinguished for definite co-ordinated movements of the different parts of the body. Those portions of the limbs, such as the fingers and thumbs, which execute delicate movements have larger areas of representation in the cortex than those whose movements are less precise. The kind of response to excitation is never predictable, however ; the same point may at one moment give flexion and at another extension. When a point is repeatedly stimulated for short periods, the resultant movement becomes larger and larger (' primary facilitation ') ; moreover, the area of cortex from which that movement can be evoked grows in size, and spreads into neighbouring areas that previously gave other responses. This is called the ' deviation of the response,' and is due to an irradiative facilitation (' secondary facilitation '). Such deviated responses may occupy large areas of the cortex.† Stimulation of points in area 6 in man gives movements in the same parts as is obtained by excitation of the adjacent portions of area 4, but stronger stimuli are needed, and it is believed by some ‡ that this result is due to transmission of the excitation to area 4. Hence an incision between areas 6 and 4 largely abolishes the effect. If the pyramidal tract has been divided, or area 4 destroyed, it is claimed, however, that strong excitation of area 6 still produces general adversive movements, *i.e.* rotation of head, eyes and trunk to opposite side.

It is claimed by Fulton, Foerster and others that area 6, which they call the *pre-motor area*, is not only connected *viâ* area 4 with the major projections of the pyramidal tracts, but also has other connections, the so-called extra-pyramidal paths, with the spinal motoneurones. Others, *e.g.* Walshe,§ regard area 6 as a region for synthesis of movements executed *viâ* the pyramidal system, and therefore as a higher level of motor control. The movements are more precise and more nearly confined to one muscle the nearer the stimulated point lies to the central fissure, *i.e.* the closer to area 4. As area 6 is approached the movement tends to become that of a whole limb.

* GRAHAM BROWN. *Q. J. Exp. Physiol.*, 1916, **10**, 103.
† GRAHAM BROWN and SHERRINGTON. *Proc. R S*, 1912, **85B**, 250.
‡ FOERSTER *Lancet*, 1931 (ii), **109**, 309.
§ WALSHE *Brain*, 1935, **58**, 49.

The movements obtained by excitation of these motor areas resemble in every respect the co-ordinated movements observed during the normal willed or spontaneous activity of the animal. Like the movements evoked they involve the reciprocal innervation of antagonistic muscles. Never do we find simultaneous contractions of antagonists, even where two opposing centres are excited simultaneously ; one reaction is prepotent, as is the case with reflexes.

Thus the movement of opening the jaw, excited from the cortex, involves a relaxation of the normal tone of the masseter muscle. Flexion of the leg demands relaxation of the extensor muscles. As in the spinal reflexes, this relaxation can be abolished under the action of strychnine or of tetanus toxin. After administration of either of these it is impossible to evoke inhibition of any muscle. Excitation of the cortical centre for the movements of the jaw then causes contraction of both closers and openers of the jaw, *i.e.* a strife in which the stronger masseter muscles predominate, so that the jaw is firmly closed.

The part played by muscular relaxation in the response to cortical stimulation is also well seen in the eye muscles. Stimulation of the centre for eye movements on area 8 of the frontal lobes on the right side causes 'conjugate deviation' of both eyes to the left. This movement involves contraction of the right internal rectus and left external rectus and a simultaneous inhibition of the tone of the right external rectus and left internal rectus. If all the nerves of the right eye be divided except the VIth, which supplies the external rectus, this eye looks permanently towards the right side, *i.e.* a right external strabismus or squint is produced. On now exciting the right cortex, *both* eyes still move towards the left, although the right internal rectus is paralysed. The movement of the right eye stops at the middle line, however, and is brought about simply by a relaxation of the tone of the right external rectus muscle. (Sherrington.)

This movement of both eyes on stimulation of one side of the brain shows that the function of each hemisphere is not entirely unilateral with regard to the muscles of the body. As a rule, the response to excitation of the motor area for limbs is only contralateral. In the case of those movements, however, which are normally carried out by co-operation of the muscles of the two sides, such as the movements of the trunk, neck, and eyes, stimulation of the motor area in one hemisphere evokes a movement involving the muscles of both sides of the body, *i.e.* the cortical representation is one of movement rather than one of muscles. Also, where an action is carried out by similar contractions of corresponding muscles on the two sides, the movement itself is bilaterally represented in the cortex. Types of such reactions are found in closure of the mouth, contractions of the abdominal muscles, extension or flexion of the trunk. It seems that under such circumstances there is a free communication between the lower motor centres of the two sides, since the bilaterality of the response is not altered by extirpation of the cortex of the hemisphere opposite to that which is being stimulated.

A certain antagonism is evident between the action of the cerebral hemispheres and that of the lower centres. Whereas in the decerebrate animal there is increased tonus in the extensor muscles, stimulation of the cortex produces flexion more easily than any other movements.

Cortical Epilepsy. When electrical excitation at any strength above the minimum effective stimulation is applied to the motor area of the cortex, the movements evoked tend to persist for a short time beyond the duration of the stimulus. This is readily understood to be due to the same causes as produce after-discharge in spinal reflexes, viz., deviation of impulse along delay-circuits. On still further increasing the strength of the current, the contraction spreads to adjoining muscles, and finally may affect all parts of the body, giving rise to an epileptic convulsion. The same effect may often be caused by weak stimuli if the irritability of the cortex be raised in consequence of previous repeated stimulation. An epileptic fit consists of two parts. The first effect of the stimulation is a strong tonic contraction ; this outlasts the stimulus for some

time, and then gives way to a series of clonic contractions, repeated at first at intervals of from six to ten per second, but gradually getting slower as the fit dies away. The tracing of such a contraction is given in Fig. 212.

The main phenomena of a fit, due to irritation of any portion of the motor area, were described by Hughlings Jackson in 1864, before the experimental proof of cortical localization had been brought forward by Fritsch and Hitzig. A similar condition may

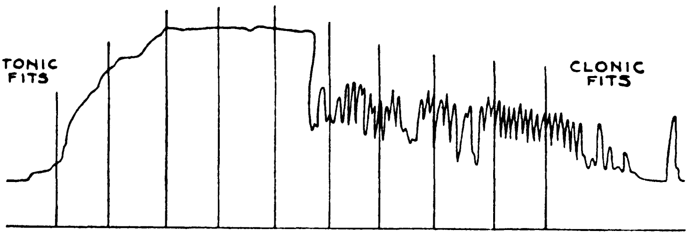

FIG 212. Tracing of Muscular Contractions during an Epileptic Convulsion aroused by Strong Stimulation of the Motor Area (HORSLEY and SCHAFER)

occur in the human subject as a result of irritative lesions of this part of the cortex, such as that due to the presence of a tumour or a spicule of bone pressing on the brain. Jackson showed that in this condition the convulsive movements follow a certain order or 'march.' Thus, if the thumb area be the seat of stimulation, the fit begins by a contraction of the thumb muscles, then spreads to the muscles of the hand, fore-arm and shoulder of the same side, and then to the face, trunk and leg. If it begins in the toes, the order would be up the leg and trunk and down the arm. The same 'march' is observed in artificial stimulation of the motor area. If the convulsions are very strong, they spread to the leg of the opposite side and then to the whole body. The spread to the other side of the body is not prevented by division of the corpus callosum, nor by isolating the centres from one another, so that the sequence seems to be maintained through the mediation of the sub-cortical centres Complete excision of the cortical centre for any given movement excludes this movement from participation in the fit. In man this type of epilepsy is, in the milder cases at any rate, generally unattended with loss of consciousness. In animals epileptic convulsions can be excited by stimulation of any portion of the cortex, though it is obtained by the weakest stimulus when this is applied to the motor cortex. Jacksonian epilepsy is often preceded by a sensation of numbness or tingling, the 'aura,' in the part in which such convulsions begin. In ordinary idiopathic epilepsy tactile or visual sensory auræ may precede the attack; but in this case loss of consciousness is always a prominent symptom, even in the milder form of the disease. During the convulsion there is a rise of blood pressure and a quickening of the pulse; the respiration is very often stopped during the tonic part of the spasm, so that the patient becomes blue The universal condition of excitation affects also the centres from which the secretory nerves originate, so that there is an excessive flow of saliva, which, in the idiopathic case, is responsible for the characteristic frothing at the mouth.

ABLATION OF MOTOR AREAS. The results of ablation of the motor areas are more serious in man and the higher apes than in lower mammals. In the dog, for instance, removal of the motor areas on one side results in nothing more than a transitory paralysis and spasticity of the musculature on the opposite side of the body.* The effect is greater and lasts longer on the fore limbs, but in a few days the paralysis nearly disappears and only a little weakness remains. The spasticity is an extensor rigidity and lasts somewhat longer than the paralysis. Bilateral removal of the motor areas is also not followed by lasting paralysis and we may take this to mean either that most of the ordinary movements and postures of the animal are reflexly controlled from lower centres, or that there are subsidiary motor centres and paths which are brought into function.

* LAUGHTON. *Amer. J. Physiol.*, 1928, **85**, 78.

In the higher apes * and in man † the results are more serious and long-lasting. Thus, if the left arm area (4b) is removed from a chimpanzee, after having its situation verified by electrical stimulation, there is at first complete and flaccid paralysis of the right arm. After a period which varies from days to weeks there is a progressive partial return of function from the shoulder downwards, the hand never recovering its finest movements completely. If the arm area on the right side is removed at this stage no effect is seen on the left arm. These observations have been interpreted by some as showing that there is some other motor centre and some path other than the pyramidal tract by which movements are controlled. Opinion is not unanimous on this, however.

When in man or one of the apes the whole of the precentral motor area (*i.e.* areas 4 and 6) are removed on one side the affected limbs on the opposite side are paralysed and their reflexes are at first depressed ; in a few days, however, spasticity may develop and extensor reflexes become exaggerated.‡ Voluntary power returns partially in a few days, but is lost again, and permanently, if the motor areas of the opposite side are removed. The posture of the animal then resembles that of the decorticate or thalamic preparation. The effects of removing area 6 (the ' premotor ' area) alone is also stated to be to cause spasticity and exaggerated extensor reflexes, with involuntary grasping movements of the hands.

These results are capable of at least two explanations. According to Walshe § the precentral motor areas comprise both area 4 and area 6, of which area 6 represents a higher level of function, largely associative in character. Spasticity only develops when the pyramidal tract is almost or completely severed from the whole area. Fulton, on the other hand, regards area 4 as the principal area of origin of the pyramidal tract, and area 6 as not only contributing to the pyramidal tract directly, and also indirectly by association with area 4, but also regards both areas, but chiefly area 6, as having other important connections with lower centres. These are called the extra-pyramidal paths and will be referred to later.

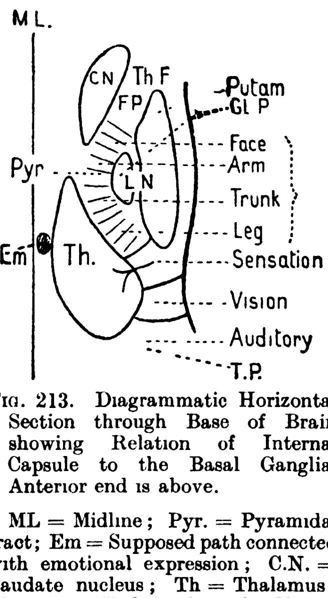

FIG. 213. Diagrammatic Horizontal Section through Base of Brain showing Relation of Internal Capsule to the Basal Ganglia. Anterior end is above.

ML = Midline ; Pyr. = Pyramidal tract; Em = Supposed path connected with emotional expression ; C.N. = Caudate nucleus ; Th = Thalamus ; Th. F. = Thalamo-frontal fibres ; F.P. = Fronto-pontine fibres ; Putam = Putamen ; Gl. P. = Globus Pallidus ; L.N. = Lenticular nucleus ; T.P. = Temporo-pontine fibres. Other words indicate position of fibres for movement of parts or fibres for sensations indicated. These fibres mostly pass vertically to plane of paper. (SAMSON WRIGHT. "Applied Physiology," Oxford University Press.)

THE PYRAMIDAL OR CORTICO-SPINAL TRACT. It has been shown that the pyramidal tract originates from the pyramidal cells in layer V of the cortex and very largely from the giant Betz cells which are found in area 4.‖ These are the only cells which show chromatolysis after section of the pyramidal tract. Some fibres are also contributed from similar cells in area 6.

The "upper neurone" fibres of the pyramidal tract pass by the corona radiata through the internal capsule, mid-brain,

* FERRIER. *Phil. trans.*, 1875, **165**, 433 ; LEYTON and SHERRINGTON. *Q. J. Exp. Physiol.*, 1917, **11**, 135 ; GRÜNBAUM and SHERRINGTON. *Proc. R. S.*, 1903, **72**B, 152.
† FOERSTER. *Brain*, 1936, **59**, 135.
‡ FULTON. *Brain*, 1935, **58**, 311.
§ WALSHE. *Brain*, 1935, **58**, 49.
‖ HOLMES and PAGE MAY. *Brain*, 1909, **32**, 1.

pons, medulla and spinal cord, to end in relation to motoneurones of the ventral horn of the spinal cord.

The *internal capsule* is a band of fibres lying between the lenticular nucleus laterally and the thalamus and caudate nucleus medially.

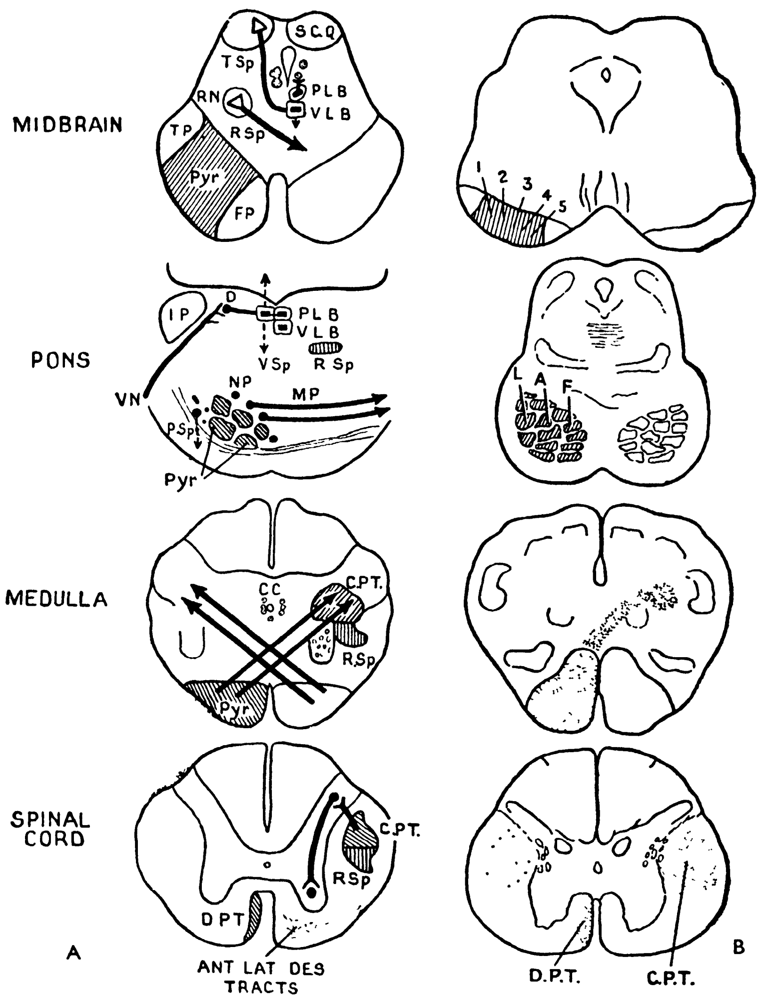

Fig. 214. A. Diagram of Pyramidal and other Motor Tracts in Man S.C.Q. = Superior corpora quadrigemina; T.Sp. = Tecto-spinal tract which passes down in V.L.B = Ventral longitudinal bundle; P L B = Posterior longitudinal bundle; R N. = Red nucleus; R.Sp. = Rubro-spinal tract; Pyr. = Pyramidal tract; T.P., F.P. = Temporo-pontine and fronto-pontine fibres; I.P. = Interior cerebellar peduncle; M P. — Middle peduncle; V.N. = Vestibular nerve; V.Sp. = Vestibulo-spinal tract; D = Deiter's nucleus, P.Sp. = Ponto-spinal tract; N.P. = Nuclei pontis, c.c. = central cord; C.P.T. = Crossed pyramidal tract; D.P.T. = Direct pyramidal tract; Ant. lat. Des. Tracts = Antero-lateral descending tracts.

B. Pyramidal tracts of ape (after Leyton and Sherrington). Stimulation at 1, 2, 3, 4, 5, gave respectively movements of leg, trunk, arm, neck and face Stimulation at L A.F. gave movements of leg, arm and face respectively. Shaded and dotted areas represent degeneration in pyramidal tract after extirpation of cortical motor area on right side. (Samson Wright. "Applied Physiology," Oxford University Press)

The position of the various motor and sensory fibres in the internal capsule is shown in Fig. 213. The motor fibres occupy the genu and the anterior two-thirds of the posterior limb, the fibres in order from before backwards being eyes, head, shoulder, arm, hand, trunk, leg, foot. They pass on as a compact mass (Fig. 214), forming the middle portion of the descending main pathway, which passes through the crusta of the mid-brain, ventral to the substantia nigra. Retaining the same situation, they reach the pons. They are here split up into discrete bundles by the cross passage of the pontine fibres. On leaving the pons and entering the medulla they form into two bundles once more. At the lower part of the medulla the main mass of fibres decussates to reach a more lateral position (the motor decussation), forming the lateral cortico-spinal or crossed pyramidal tract (about 10 per cent. of its fibres are not crossed however). A remnant of fibres, which is called the anterior cortico-spinal, or direct pyramidal tract, is left behind, close to the anterior fissure. Both direct and crossed pyramidal tracts descend in the cord in these two situations, turning in at different levels to relay at anterior horn cells, the crossed ones to cells of the same side, the uncrossed ones to cells of the opposite side. In this way fibres that have originated in the precentral motor area on the one side of the brain innervate anterior horn cells which send fibres to muscles on the other side of the body.

Various collateral paths are formed from the pyramidal tracts. The chief of these are to the nuclei pontis, from which fibres pass in the middle peduncle to reach the cerebellum on the opposite side. Other branches of the tract cross in the mid-brain, pons and medulla to reach the motor nuclei of cranial nerves on the opposite side.

OTHER MOTOR AREAS. Movements can be excited by stimulation of various other cortical areas in addition to the precentral ones described above The chief of these (Fig. 210) are :—

(1) Various areas in the lower part of area 6 cause movements of mouth, larynx, arrest of breathing, etc.
(2) The so-called 'frontal eye-field (area 8) causes conjugate deviation of eyes and head to the opposite side.
(3) Area 19 in the occipital region gives constriction of pupil, and, if strongly stimulated, conjugate deviation of eyes to opposite side.
(4) The post-central area (3–1–2) in the parietal lobe yields movements similar to those obtained from corresponding levels of area 4. These results in man are accompanied by sensations in the parts served, and are no doubt due to irradiation to area 4.
(5) Area 5 of parietal lobe and area 22 of temporal lobe may also cause adversive movements of head and eyes, also probably a secondary effect of sensory stimulation.

Of these areas the frontal eye field is the most definitely related to voluntary movements, since ablation of that area leads to impairment of eye movements. The other areas are perhaps largely reflex in function and concerned with postural reflexes and such automatic acts as respiration, pupillary constriction, vocalization, etc.

The Extra-pyramidal System. The evidence obtained from ablations of the precentral cortex in man and the higher apes, together with other clinical evidence, points to there being other paths besides the pyramidal tracts by which the cortex is connected with lower motor centres. Thus, the eye movements controlled from area 8 in the second frontal convolution, and the complex movements of body and limbs obtained from area 6 of the precentral area, are obtainable after section of the pyramidal tracts. These so-called extra-pyramidal tracts still remain somewhat obscure, but those for the trunk and limbs appear to have important connections with the basal ganglia. The simplest of them is the path from the frontal eye field, called the *cortico-nuclear* tract. The fibres pass in the anterior limb of the internal capsule, with the fronto-pontine fibres, thence in the mesial fifth of the crusta of the mid-brain, from which fibres pass dorsally to the oculomotor nuclei of the opposite side.

The other cortical projections have a more complicated pathway. They are claimed to arise from all the motor areas, which may include some extra-pyramidal projections, in addition to pyramidal ones, *e.g.* from the precentral areas, and especially from area 6.

Thus after section of the pyramidal tracts, a flaccid paralysis results, and stimulation of area 4 then gives movement, or, more usually, an inhibition of any postural tonus.* Stimulation of area 6 gives complex movements. It is claimed that when spasticity results from removal of cortical areas it is a release phenomenon due to the severing of extra-pyramidal connections † which normally exercise a controlling influence.

The efferent cortical paths, including the important extra-pyramidal ones, are shown diagrammatically in Fig. 215.

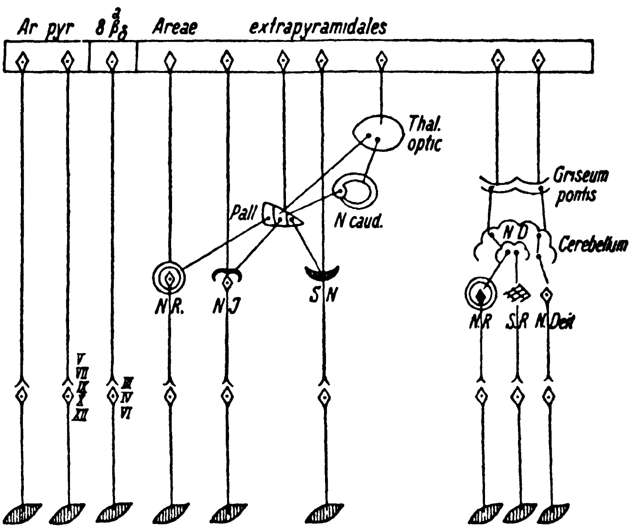

FIG. 215. The Pyramidal and Extra-pyramidal Cortical Projections Schematized. Ar. pyr. = The precentral cortex from which the pyramidal tracts arise; 8 = The frontal eye field: areæ extrapyramidales, the extrapyramidal cortex, Thal. optic = Thalamus; N. caud = Caudate nucleus; Pall. = Globus pallidus; N.R. = Red nucleus; N T. = Tegmental nuclei; S N. = Substantia nigra; S R. = Reticular formation; N Deit = Deiter's nucleus. III to XII, Motor nuclei of cranial nerves. (After FOERSTER, *Brain*, 1936, **59**, 135.)

It will be seen that there are important connections with the basal ganglia.

THE BASAL GANGLIA include:

(1) The corpus striatum.
  (i) Caudate nucleus.
  (ii) Lenticular nucleus.
    (*a*) The putamen.
    (*b*) The globus pallidus.
(2) The claustrum.
(3) The amygdaloid nucleus.
(4) The body of Luys.

The relations of the lenticular nucleus are shown in Figs. 213 and 216.

The *internal capsule* through which the principal cortical fibres pass on their way to or from the brain stem is placed between the caudate nucleus and the thalamus mesially, and the lenticular nucleus laterally. Between the lenticular nucleus and the more laterally placed claustrum is another band of white fibres, the *external capsule.*

The thalamus, as we have learned in Chapter XIX, is a sensory relay and association centre. The *corpus striatum* is believed by some neurologists to be a group of stations placed on the efferent path. It represents the remains of a phylogenetically old motor

* MARSHALL. *Arch. Neurol. Psych.*, 1934, **32**, 778, TOWER and HINES. *Science*, 1935, **82**, 376.

† FULTON. *New Engl. J. Med.*, 1937, **217**, 1017.

centre, now largely supplanted in the higher animals by the efferent portions of the cortex.

The connections of these nuclei are complex and not yet fully worked out, but it seems clear that the *globus pallidus* still has motor functions. The putamen and caudate

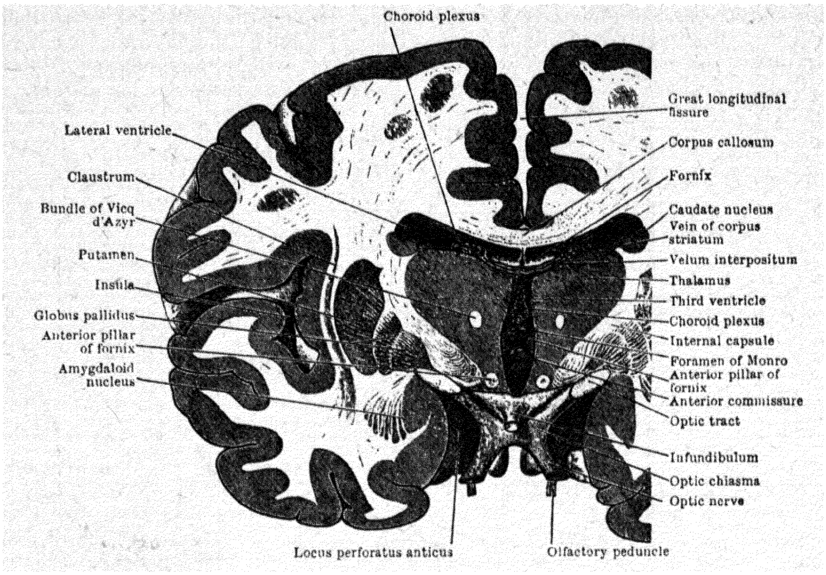

FIG. 216. Coronal Section through the Cerebrum so as to cut through the Lenticular Nucleus; Posterior Surface of the Section depicted. (CUNNINGHAM.)

nucleus have associative connections with the frontal lobes and with thalamic and hypothalamic nuclei, but their only efferent projections are to the globus pallidus.

The *globus pallidus* contains large multipolar cells of an efferent type. It receives fibres from the putamen and caudate nucleus, and a few from the cerebral cortex. It sends out *viâ* the ansa lenticularis fibres to many parts * the chief of which are :—

(1) The body of Luys.
(2) The hypothalamus.
(3) Oculomotor and most other motor cranial nuclei.
(4) Red nucleus (mesencephalon).
(5) The substantia nigra.

There may thus be another, though indirect, efferent motor path from the cortex, in addition to the cortico-spinal tracts already discussed. Thus from cortex to thalamus or caudate nucleus, thence to the globus pallidus, from which relay fibres lead to the bulbar nuclei and reticular formation of the brain stem, the red nucleus, substantia nigra, etc. Thence the spinal cord may be reached by reticulo-spinal, tecto-spinal, olivo-spinal and rubro-spinal tracts.

Kinnier Wilson † has pointed out that destruction of the corpus striatum in man may give rise to a condition (Parkinson's disease), characterized by tremor in the execution of willed movements and increased tonicity of the muscles. He therefore ascribes to these bodies, or rather to the sensori-motor mechanism which has its chief meeting-place in their nuclei of grey matter, a steadying effect on the motor system, and places this system by the side of the vestibular, the cerebellar and the pyramidal systems

LESIONS OF THE MOTOR PATHS IN MAN

The pyramidal path comprises three neurones. The first called the *upper motor neurone*, starts in a Betz cell of the cortex and ends at the second, an intercalated neurone in the dorsal horn of the cord, which passes

* MORGAN *Arch. Neurol Psych.*, 1927, **18**, 495.
† WILSON. *Arch. Neurol. Psych.*, 1924, **11**, 385.

on to a motoneurone in the ventral horn, and this third, or *lower motor neurone,* comprises the spinal motoneurone and its axon which enters one of the motor nerves. It has long been recognized by clinicians that the effects of section of the upper and lower motor neurones differ greatly. Lower motor neurone lesions, *e.g.* by cutting the motor nerve, or by destruction of the motor nerve cell bodies, as in the results of poliomyelitis, produce permanent flaccid paralysis and subsequent wasting of the muscles.

Lesions of the upper motor neurone as a rule produce quite other results. If the lesion is in the cortex the result will depend upon its position and extent. These have already been considered ; the main point is that at least partial recovery occurs, but spasticity appears and persists if the lesion is extensive. The commonest site for lesions of the upper motor neurone in man is in the internal capsule, the cause being thrombosis in or rupture of the small arteries that supply it (apoplexy). The result, if on one side, is a paralysis of the muscles of the opposite side, the situation depending on the actual site and extent of the lesion. Often it is a general paralysis of one side (hemiplegia), the right-sided paralysis due to a left-sided lesion being accompanied by loss of speech. Respiration and other involuntary movements, *e.g.* some postural reflexes, are little affected, and muscles that act on both sides, *e.g.* eyes, trunk, etc., suffer little loss of function.

At first, in hemiplegia, the paralysed muscles are flaccid, but after a stage of shock has passed off, certain muscles become hypertonic (spastic). The knee-jerk is more sustained than normal, a Babinski plantar reflex is obtained, and characteristic limb attitudes result. These are similar to the ones described under decerebrate rigidity (p. 289), and the explanation is no doubt similar, viz. that the rigidity is due to release from some higher control.

It is difficult to see why the upper motor neurone lesion should give a state of tonus so different from that resulting from lower motor neurone lesions, unless we admit that there is also damage to neurones other than those of the pyramidal tract and which also play upon the final common path of the lower motor neurones. This is generally admitted for the condition of decerebrate rigidity, and in totally decorticate animals, etc., where quite complex motor reflexes can be carried out. Mere section of the pyramidal tracts apparently leads only to a flaccid paralysis,* whereas destruction of the motor path in the internal capsule by a hæmorrhage results in a spastic condition. We must suppose, therefore, that the spasticity is the result, not of the interference with the pyramidal path, but of damage to the extra-pyramidal paths which run near to them, and which normally inhibit that overaction of lower centres which in its most developed form is recognized as decerebrate rigidity.

THE PART PLAYED BY AFFERENT IMPRESSIONS IN THE CO-ORDINATION OF MUSCULAR MOVEMENTS. In the carrying out of the muscular movements of the limbs, impulses are set up in the proprioceptive structures, including those of muscles. These secondary afferent impulses in their turn affect the excitability and the activity of the motor neurones, and are important in regulating the movements, whether these be aroused by immediate sensory stimulation of the surface of the body, or through the higher parts of the brain, as in volitional movements.

Their significance is shown by the marked disorders of movement produced in a limb by section of its afferent nerves. Thus, if all the posterior roots

* MARSHALL. *Arch. Neurol. Psych.*, 1934, **32**, 778 ; TOWER. *Brain*, 1935, **58**, 238 ; 1936, **59**, 408.

supplying one hind limb of the frog be divided, the posture of the desensitised limb is abnormal, whether the frog be suspended or be in a sitting posture. Such a frog generally swims with the desensitised limb in permanent extension. The complete absence of muscular tone under these circumstances has already been mentioned. When, in a mammal, a contraction of the quadriceps extensor is induced by a single shock applied to the intact ant. crural nerve, the curve obtained shows a relaxation time much slower and more prolonged than when the cut nerve is similarly excited. In the latter case, or when the posterior roots alone are divided, the lever at the end of relaxation dips below the base line with an inertia fling, which is never present when the nerve is intact. The contraction of the muscle, when its afferent path is intact, seems to develop reflexly in the muscle itself a condition of tone which damps the inertia swing of the contraction. In the dog, after section of the afferent nerves of one hind limb, this limb is not at first used for walking; it is kept more or less flexed at hip and knee, and later, when it is employed in walking, it is lifted too high with each step. After division of the afferent fibres of both limbs, these appear as if they were affected with motor paralysis. At first during walking, the fore limbs simply drag the hind limbs after them, though later, as the hind limbs are drawn along, they make alternate movements and may ultimately afford a certain amount of support to the body.

Still more striking effects are observed in complete deafferentation of the fore limb in monkey or man. The limb is never used in climbing, nor in the taking of food. That the peripheral motor mechanism is intact is shown by the fact that stimulation of the appropriate area of the cerebral cortex in such animals elicits at once a movement of the hand or limb. It seems, however, impossible for the cortex to *initiate* such movements in the absence of all afferent impulses arriving from the limb.

In these phenomena of sensory paralysis we are dealing with the effects produced by the deprivation of two distinct classes of afferent impressions, viz. those from the skin and those from the deep structures and muscles. The phenomena due to these two factors may be studied separately. If, in the monkey, all the afferent brachial roots except the last cervical, which supplies cutaneous sensations to the whole hand, be divided, the monkey uses the arm and hand both in climbing and in taking food. A marked ataxy of the movement is, however, observed. Whereas the normal monkey, in taking grains of rice out of the observer's hand, exhibits perfect precision of movement so that he rarely touches the hand on which the grains are lying, the monkey with only cutaneous sensibility remaining grasps clumsily with the whole hand, and the arm sways as it is put out, often missing the object aimed at altogether. These effects are due to the loss of sensations from the deep structures and muscles. On the other hand, cutaneous insensibility of a limb of an animal causes very little disturbance of locomotion, the alternate movements of which seem to be started by the stimulation of deep structures and muscles.

The impressions from the muscles play a more important part. Division of the afferent nerves from the muscles gives rise to a condition of tonelessness, and the passive mobility of the joints is greater than usual, so that the hip with the limb extended at the knee may be flexed to an abnormal extent. The disturbance of co-ordination resulting from the cutting off of afferent muscular impressions is well seen in cases of tabes dorsalis (locomotor ataxy) in man, and to a slighter extent in cases of peripheral neuritis affecting chiefly the sensory nerves of muscles. The ataxic gait of such a patient is characteristic. There is no loss of power in the muscles, but there is loss of control. The patient is unaware of the position of his limbs and has to guide

his walk by visual impressions : even then the movements are inco-ordinated. The contraction of every muscle is exaggerated, so that in walking the leg is first raised too high and then is brought down on to the ground with a stamp.

Such a patient may show 'static ataxy,' *i.e.* he is unable to stand with his feet together and his eyes shut. The afferent impressions from the skin of the feet and from the muscles appear therefore to be necessary also for the maintenance of static equilibrium. As the disease progresses the loss of control becomes more and more pronounced, so that attempts to walk simply give rise to a profusion of disordered movements, the legs being thrown in all directions with the patient's efforts, but with no effective result. The centres are no longer informed of the degree to which each muscle is contracted, and the impressions are wanting which should cut short the contraction of a muscle when it has attained its optimum and which should inhibit the antagonists during the contraction and induce activity of the antagonists in successive alternation to those of the other muscles. In such a patient, therefore, walking finally becomes impossible, and, with well-nourished muscles and a motor path which is intact, he is condemned to pass the rest of his days in bed.

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CHAPTER XXI

FUNCTIONS OF THE CEREBRAL CORTEX

From the evidence which has been reviewed it may be inferred that parts of the cerebral cortex act as final receiving stations for the afferent impressions from all parts of the body, including the organs of special sense, while other parts act as stations of origin for motor and other efferent impulses. A simple view of one of its functions would be that it acts as a centre in which, as in the spinal reflex centres, afferent impulses are reflected outwards as motor impulses. But we know also that the cerebral cortex is the seat of all the higher activities of the mind. In particular the frontal areas are concerned with these associative functions. The study of these belongs to the province of psychology, which employs methods different from those of physiology for its approach. Accompanying the passing-over of impulses from the afferent to the efferent side of the cerebral arc, it would seem that the state which we call consciousness is engendered. The states of consciousness glide continually from moment to moment in an unbroken stream of experience, consisting of a sharper focal content with a fringe of slighter definition, and leaving behind it a trace which we know as memory. By a process of attention we can single out parts of the stream of consciousness for closer focussing.

There seems but little doubt that our conscious experiences are the result of complex integrations of sensory impressions, which are assessed by being checked and compared with traces of previous experiences. Around each specific sensory receiving area is an associative zone, the function of which is believed to be concerned with the perception of the sensations aroused. Thus the visual sensory area is the area striata, Brodman's area 17, and its stimulation leads to the production of sensations of light. Around it are areas 18 and 19, where these sensations are tested and integrated with tactile or other sensations, so that out of the process visual perceptions are arrived at. Stimulation of these areas leads to hallucinations of definite objects, *i.e.* the sensation is intellectually classified and recognised.

There are various objective methods by which the physiology of cerebral activity may be studied, and they are of different significance. Among others, we have the study of reaction times, electroencephalograms, animal behaviour, conditioned reflexes, and the varied phenomena of disease.

REACTION TIME. In studying reaction times we measure the time interval between the application of a stimulus and a voluntary response. The greater the number of synapses involved in any neural reaction, *i.e.* the greater the complexity of the reaction, the longer will be the period which elapses between the moment of application of the stimulus and the moment at which the response takes place. Especially is this the case when the complex meshwork of neurones of the cerebral hemispheres is involved. The determination of the *reaction time* gives information as to the time relations of the psychical processes which are the representation in consciousness of the physiological changes occurring in the neurones of the central nervous system.

In most methods the application of the stimulus is arranged so as to close the circuit of a current which flows through an electro-magnet activating a lever which writes on a rapidly moving blackened surface. The reaction of the individual who is the subject of experiment is arranged so that the resulting movement activates a key by which the same current is opened (Fig. 217). We thus obtain a tracing on the blackened surface showing the moment of application of the stimulus and the moment

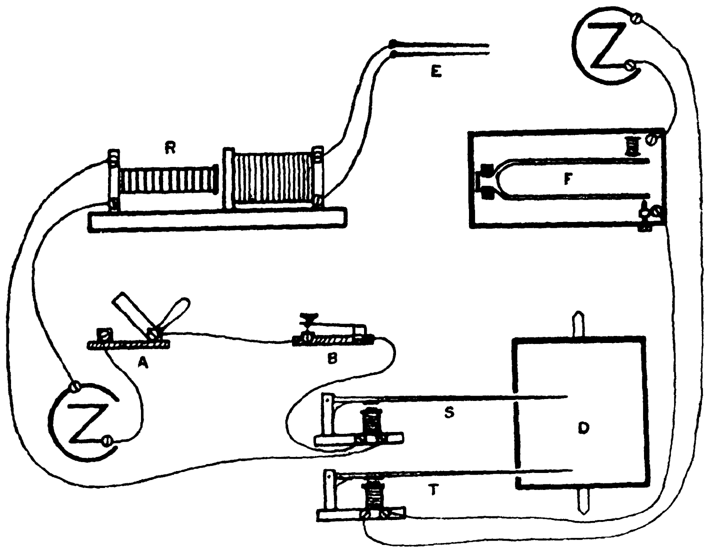

FIG. 217. Arrangement of Apparatus for Determination of Reaction Time for Touch (ALCOCK and ELLISON)

R, coil; E, exciting electrodes; F, tuning-fork; A, B, keys, S, T, electro-magnetic signals, D, drum

at which the reaction takes place. Thus, if the reaction time for an auditory stimulus is to be determined, the electric current is arranged so as to pass through:

(1) A spring contact key which can be pressed so as to make a sound, *e.g* to ring a bell.

(2) An electric signal writing on a rapidly moving surface.

(3) A second key which the subject will release as soon as he hears the sound of the signal, and so break the current.

If the sensory impression is to be from the skin, the current may be made to pass through the primary circuit of an induction coil, and wires be taken from the secondary coil to some part of the surface of the skin (Fig. 217). In this case the signal may be started by opening the circuit, and the subject of the experiment will respond by closing the circuit by means of a spring key (B) directly he feels the shock caused by the break of the primary circuit. If the reaction period is to be determined for sight, a piece of white paper may be placed on an electro-magnet in the primary circuit and the person will respond directly he sees this move.

The average reaction times in man obtained with the different senses are as follows:—

Sight.	Hearing.	Electrical Stimulation of Skin.
0·19 to 0·22 sec.	0·12 to 0·18 sec.	0·12 to 0·20 sec.

The two figures given for each case are the extremes obtained in different series of observations.

The times vary according to the condition of the person who is the subject of the experiment. They are lengthened by fatigue; they are shortened up to a certain point by continued practice. Within limits also they are shortened by increase of the strength of the stimulus.

DILEMMA. When the subject is required to distinguish between alternative stimuli, the reaction time is considerably longer. Thus, the wires from the secondary coil may be connected by a switch to two pairs of electrodes which are applied, one to the right and one to the left half of the body. It is agreed beforehand that the subject shall react, say, only to stimulation of the right side. The switch is removed from the observation of the subject and the stimulus is applied irregularly to one side or to the other. It is found that the additional neural processes involved in determining whether the stimulus is on the right side, and therefore should be followed up as agreed, adds considerably (on an average 0·06 sec.) to the length of the reaction time. It is possible to complicate the dilemma to almost any extent. Thus the experiment may be so arranged that either a red or a white disc appears, and the subject has to react with the right hand to the red disc and with the left hand to the white disc. In such an experiment the reaction time was found to be 0·154 sec. longer than the simple reaction time. A still more complex process would be involved in the experiment in which a word was spoken, and the subject had to speak some other word which had some association with the word which formed the stimulus, *e.g.* horse—mammal; paper—pen, &c. In such an experiment the reaction time was found to be as long as 0·7 to 0·8 sec.

We see that the recording of the exact moment of occurrence of any physical event can occur only after a certain lost time, which represents the observer's reaction time for the stimulus in question. This applies, however, only to movements carried out in response to isolated stimuli, or to stimuli repeated at irregular intervals. When the stimuli are rhythmic the lost time applies only to the first one or two of the stimuli. The observer is conscious of the interval elapsing between the physical event and his reaction, and anticipates the later stimuli so that his reaction becomes synchronous with the stimulus. This synchronism of stimulus and reaction characterises all rhythmic movements, such as dancing or the playing of an orchestra

ELECTRICAL ACTIVITY OF THE CORTEX. When the cerebral cortex of an anæsthetised animal is led off by two electrodes placed on its surface and connected with a suitable amplifier and recording oscillograph, potential waves may be recorded.* These are roughly divisible into large slow waves, at 1–4 per sec., and superimposed smaller waves. These waves are the resultant of large numbers of short waves in individual neurones, the response of each unit probably varying in duration according to conditions, from 0·1–0·01 sec., but of uniform size. Increasing or reducing the anæsthesia may alter the magnitude of the waves; injury causes a rapid series of waves, the frequency of which gradually dies down from 100 to about 40 per sec., and which appear to be due to synchronous pulsation in large groups of neurones (Fig. 218).

The arrival of afferent impressions at the cortex results in the starting off of trains of potential waves which originate from the special sensory area concerned. Thus if light falls on the eye, rhythmic discharges spread out from the area striata,† and so on. It is not certain whether these waves are initiated in the afferent terminal fibres or in internuncial cells affected by them. They show fluctuations according to the general background of cortical activity on which they impinge. The rhythmic potential waves of this background tend to be increased locally on arrival of an afferent stimulus

* ADRIAN and MATTHEWS. *J. Physiol.*, 1934, **81**, 440.
† CLAES. *Arch. internat. Physiol.*, 1939, **48**, 181.

and this effect resembles that of direct electrical stimulation of the cortex.* Electric stimulation of the cortex starts waves which radiate out over the cortex, the size of wave and the extent of spread increasing by facilitation

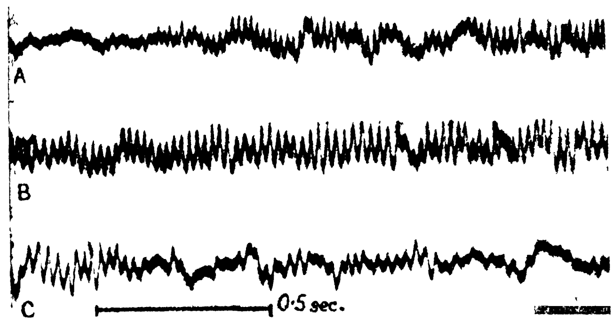

FIG. 218. Injury Discharge from Cortex of Anæsthetised Rabbit, punctured with fine Wire. A. Beginning, frequency = 64 per second ; B. Middle, frequency = 44 per second : C. End, frequency about 40 per second. (ADRIAN and MATTHEWS, *J. Physiol.*, 1934, **81**, 440.)

as the stimuli are repeated. As the stimuli are repeated at the same spot, also, it is found that the point from which the waves begin tends to drift to other positions as other neurones, by facilitation, become more excitable. After further repeated stimuli, in fact, the whole cortex may be involved and may be driven as by a pacemaker, from the area of origin, and at its rhythm. If two centres compete in the emission of cortical waves the regular rhythm may break down into small rapid and irregular oscillations. Centres of inhibition may also be set up and the inhibitory influence may likewise irradiate widely over the cortex.

Severe injury or convulsant drugs cause the pulsations to spread widely over the cortex and in different directions, and large irregular waves are often seen.

Somewhat different potential waves (electroencephalograms) can be detected when electrodes are applied to the human scalp.† The chief are the *alpha waves* (Fig. 218). These occur at about 10 per second ; they usually arise in the visual area of the occipital lobe.‡ They are present when the eyes are shut or when, with uniform visual fields, there is no attempt to see detail, and are reduced or abolished whenever the central field of vision has any detail, or in any mental state, visual or otherwise, which demands attention. It is significant that the latent period for disappearance of the α waves on opening the eyes is about 0·4 second, *i.e.* it is longer than the motor reaction time.

Adrian and Matthews regard these waves as due to spontaneous rhythmic activity arising simultaneously in groups of neurones in an area of visual cortex when this is not engaged in definite function.

Sometimes waves at about 25 per second also appear. These, the *beta waves*, possibly originate in the sensori-motor areas. They tend to be inhibited by tactile stimuli. In sleep there are sometimes intermittent

* ADRIAN. *Proc. R. S.*, 1939, **126B**, 433.

† BERGER. *Arch. f. Psychiat.*, 1929, **87**, 527 ; 1933, **101**, 454 ; KREEZER. *Amer. J. Psychol.*, 1938, **51**, 737.

‡ ADRIAN and MATTHEWS. *Brain*, 1934, **57**, 355.

'bursts' of waves at 14 to 15 per second, which are called 'spindles,' and in deep sleep a slow random rhythm, the *delta waves*, at about 6 a second.

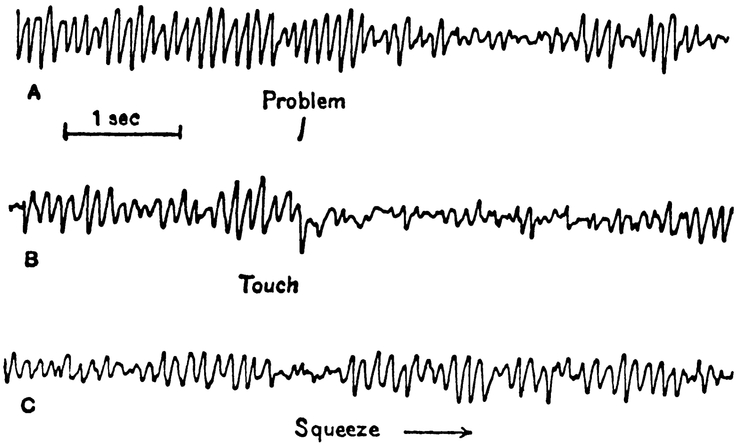

FIG. 219. 'Berger' rhythm from Human Scalp. A and B show depression of waves by non-visual activities. At A, a mental arithmetic problem. At B, a touch on nose with cotton wool. C. Squeezing pliers tightly shows that muscular efforts do not affect rhythm greatly. (ADRIAN and MATTHEWS, *Brain*, 1934, **57**, 355.)

It is probable that these various rhythms are not due to the effects of afferent impulses arriving at the brain, but represent a tendency on the part of groups of nerve cells to emit regular synchronous potential waves.

This property is seen in almost any isolated portions of grey matter. Thus the isolated medullary centres of the goldfish show potential waves at a rate corresponding to that of the normal respiratory movements,* and portions of the frog's brain, especially the olfactory lobes, also show characteristic rhythms when isolated.†

In the brain *in situ*, moreover, it was found by Bremer ‡ that section across the mid-brain did not cause the cortical rhythms to disappear, although all afferents except olfactory and optic were removed thereby. These facts are of great importance inasmuch as they indicate that even in absence of stimuli from without, the cells of the cortex are not inactive, but tend over whole areas to fall into synchronous rhythms, the corresponding foci in the two hemispheres being also synchronous. There are some indications that mutual reinforcement by the cortex and the thalamus is largely responsible for maintaining the spontaneous rhythm.

Animal Behaviour. Another objective method of investigation is by the study of the behaviour of animals as affected by conditions such as the extirpation of cortical areas. The methods mostly depend on the use of tests which involve the learning of something. Thus rats can learn to traverse a maze in response to a particular stimulus, *e.g.* light, or monkeys can acquire skill in unfastening latches, &c. After removal of the entire cerebral hemispheres nothing can be learnt, and even complex behaviour generally classed as instinctive, *e.g.* nesting of birds, may be lost. It is often supposed that when a habit has been acquired by voluntary effort, it ultimately becomes a reflex in which only the lower centres are concerned.

* ADRIAN and BUYTENDIJK. *J. Physiol.*, 1931, **71**, 121.
† GERARD and YOUNG. *Proc. R. S.*, 1937, **122**, 343.
‡ BREMER. *C. R. Soc. Biol*, 1935, **118**, 1235, 1241.

The truth of the matter seems to be that cortical and sub-cortical processes react considerably one upon the other, and that many apparently cortical responses are really mediated by sub-cortical structures, though removal of the cortex does nevertheless modify or abolish such responses. Examination of animals in an ascending evolutionary scale shows that the cortex takes over to an increasing extent the functions of older structures, so that conclusions based on lower species are not necessarily applicable to higher ones.

One of the most striking results of animal behaviour experiments is the demonstration by Lashley that the effects of removal of cortical areas can be counteracted by training or retraining. Thus rats can be trained to enter an illuminated alley and avoid a dark one. If, after training, the striate areas are removed the rat fails to pass the test and will never spontaneously recover the ability. Removal of up to 60 per cent. of other parts of the cortex leaves the ability unimpaired. Animals with striate areas destroyed can, however, be trained as quickly as normal rats to pass the test, and retraining of those who lost the ability by removal of the striate area also restores the ability. Such experiments, and similar ones on auditory centres, show that the idea of a simple path from receptor to cortex is quite inadequate.

Similar vicariation of function is shown in the motor paths of the cortex, and it has been found possible to train movements after removal of the motor centres primarily concerned with them, or after destruction of pyramidal tracts.

It may be concluded from experiments along these lines that there is less precision of localization of functions in the cerebral cortex than the earlier work on localization led us to expect, and that there seems to be a certain amount of mutual dependence between parts of the cortex. While there are certain fairly precise fields of local representation, or of special function, there appear also to be wider and less precisely defined areas between which there is some latitude with respect to function.

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CONDITIONED REFLEXES

Pavlov introduced a method for investigating the functions of the higher centres, which is capable of very wide applications. It is not open to the same objections as older methods, since it is applied to perfectly normal animals, and affords objective means of observation of the effects and interaction of sensory stimuli.

THE UNCONDITIONED AND THE CONDITIONED REFLEX. All reflex activities can be divided into two large groups: the *inborn*, and the *acquired* reflexes.

The inborn reflexes are characteristic of the central nervous system of any whole class of animals: they are transmitted by heredity, and their formation is therefore independent of the conditions under which any individual animal may live. Their strength may vary in the course of the animal's life: some of them do not appear until a definite age is reached, some disappear in senility, but in each case always to reappear in the next genera-

tion. To express the permanent stable character of these reflexes and their slight dependence upon surrounding conditions, Pavlov gave to the inborn reflexes the name of *unconditioned reflexes*. All spinal and bulbar reflexes are unconditioned reflexes : the knee jerk, the stepping reflex, the secretion of saliva caused by administration of food, the flying reflex of birds, most of the postural, vascular and lower sexual reflexes, are included in the group of unconditioned reflexes. Some of these reflexes may be extremely complex and involve a series of successive reflexes, or be of an elaborate integrative nature, forming chains of reflexes following one another and presenting different groupings. What is generally known under the rather ill-defined name of 'instincts' is probably nothing but a very complex integration of these inborn or unconditioned reflexes.

The acquired reflexes are peculiar to a single individual animal and may be entirely absent from the rest of the individuals of the same class. They are acquired in the course of the animal's existence, as a result of adaptation to changes in the surroundings. The organism at birth presents merely greater or lesser possibilities for the formation of these reflexes, but their actual formation depends only on the conditions under which the individual animal lives. They disappear more or less quickly when the conditions of the animal's life become so changed that it has no more use for the previously acquired reflexes ; instead of the lost ones it forms new reflexes. Hence the name given to them of *conditioned reflexes*. However, it is important to remember that the conditions of life among individuals of the same class of animals may be identical in many respects ; therefore it is not surprising that, besides possessing all the inborn unconditioned reflexes, these animals will also have many conditioned reflexes in common. The following example may serve as an illustration. Meat when placed in the mouth of any dog causes a reflex secretion of saliva and a reflex act of deglutition. It is common knowledge that salivation will also be produced if meat is only shown or given to smell. The latter reflex will be found just as universally amongst the carnivorous animals as the former. Food therefore, besides its property of causing these reflexes when directly stimulating the taste, can cause a similar effect through the optic and the olfactory nerves. Only by means of special experiments was it possible to show that secretion caused by the application of the stimulus to the taste buds is based on an unconditioned reflex, while that excited by the *stimulation of the visual and olfactory* receptors is a conditioned, *i.e.* acquired, reflex, and is common to all *carnivorous animals only because the conditions of their life are identical in respect of their food.*

THE FORMATION OF CONDITIONED REFLEXES. Conditioned reflexes never originate spontaneously. They only develop in association with another previously established reflex. In the simplest case conditioned reflexes are based upon an inborn reflex and, since conditioned reflexes are not hereditary, we must look upon all conditioned reflexes as being an associative development of the inborn reflexes which ultimately lie at their root. Endless numbers of conditioned reflexes can be based upon one single unconditioned reflex. Conditioned reflexes of the second, third and higher orders can be developed in association with the primary conditioned reflexes, thus forming a complicated collection of nervous activities which are governed by education, experience and adaptation to the secondary conditions. The higher the animal's nervous system the greater the number and complexity of the conditioned activities it presents.

The experimental study of conditioned reflexes has dealt up to the present

mostly with the simplest kind of reflexes which are developed in association directly with the unconditioned reflexes (conditioned reflexes of the first order). When a stimulus which is quite neutral, in that it does not by itself originate any kind of reflex activity of the animal, is repeatedly used during the execution of an inborn reflex (this latter of course in response to another stimulus), it loses its neutral character and acquires the property of bringing about by itself the same result as the inborn reflex. The hitherto neutral stimulus is transformed into *a conditioned stimulus* : it acquires the properties of the stimulus which initiated the reflex, and this can now be set into operation by the new accessory stimulus.

An example will illustrate the point. It is well known that food or acid when placed in an animal's mouth causes a reflex secretion of saliva, which in a dog with a salivary fistula can be collected and measured. This secretion is an inborn reflex, and does not require the presence of the cortex, since it can be observed after decerebration or even on a bulbo-spinal preparation. Now if a second stimulus, say a sound (or any other stimulus), is applied to the animal simultaneously with feeding and this combined stimulation is repeated several times, we find that the sound acquires stimulative properties and, when used alone without feeding, causes a secretion of saliva. A new reflex has thus been formed and a new nervous association established. That sound in respect of the salivary gland becomes a signal for the food. Any other effector organ of the animal besides the salivary gland, or any sort of stimulus, can be used for the establishment of conditioned reflexes. On account of the comparative simplicity of measuring the reflexes the salivary glands have been most used as effectors. Conditioned reflexes have been established and studied also in the case of striated muscles, respiration, vasomotor system, pupil, deglutition, excretion of milk, contraction of the bladder, and intestinal and gastric secretion. It has been found possible to establish reflexes to visual, auditory, tactile, thermal, kinæsthetic, and olfactory stimuli.

RATE OF DEVELOPMENT OF CONDITIONED REFLEXES. Experiment shows that the development of conditioned reflexes is comparatively rapid. For example, a conditioned reflex was developed between the showing of food and the sounding of a note of 637·5 vibrations per second. The table shows that the reflex had been established by the thirty-first repetition.

Number of repetitions of combined stimuli	Strength of the conditioned reflex when sound tried alone Drops per 30 secs	Latent period of the secretion in secs.
—	0	—
9	18	15
15	30	4
31	65	2
41	64	3
51	69	2

Such a conditioned reflex as the above might be established within a few days, by repeating the combined stimuli of food and sound many times per day. When once such a conditioned reflex has been established, evidence of its persistence may be obtained for many years afterwards. If, however, the experiments be discontinued before it has been

fully established the effects will be of a temporary nature. Suppose, for example, the above experiment had been discontinued on the ninth day, then, if tested on the fifteenth day, with no intermediate experiments, the probability would have been only a small secretion of saliva, indicating that the effect hitherto produced was fading away. If, on the other hand, the trials were continued until the fifty-first experiment, then a considerable effect might be obtained many months later. This forms the basis of 'ingrained habits.'

LAWS OF CONDITIONED REFLEXES. Since the phenomena connected with conditioned reflexes are almost always investigated by means of some easily observed reflex act, such as salivation, the idea is apt to arise that conditioned reflexes only concern phenomena of this type. Such an idea is, of course, entirely erroneous. Such reflexes are chosen because by their use the observer has a simple and objective indication of what is happening in the animal's nervous system.

SUMMATION. If, by means of separate applications, conditioned reflexes have been set up for two different kinds of stimuli, then, on subsequently applying both of them together, a greater effect may be produced than either would give alone. For example, suppose a conditioned reflex be set up by giving food and by sounding a whistle, and also in another series of experiments, carried on at the same time, by giving food and stimulating the back electrically, then, if subsequently the electrical stimulation is given and the whistle sounded together, the number of drops of saliva will be found to be the sum of those given by either stimulus alone.

IRRADIATION. Suppose that a conditioned reflex be established for a carefully-marked area of skin, and that by testing this area the strength of the conditioned reflex be measured. If now a slightly different area of skin be stimulated, it will probably be found that there is still a flow of saliva, though a smaller one than that obtainable if the correct spot had been stimulated. As spots further and further away from the correct one are tried, the number of drops decreases. A spreading of effect of this kind is called irradiation.

SPECIFICITY. Suppose that, having established a conditioned reflex for a particular gong, a gong of different pitch be sounded, then there may be either no flow of saliva or quite a considerable flow, according as the difference in pitch between the two gongs be perceptible or not. This phenomenon is called specificity.

DISCRIMINATION. Suppose that a series of gongs of different pitch are obtained and that two of them be experimented with in the following manner. The sounding of gong A is always accompanied by the giving of food, whereas the sounding of gong B is not. Then on subsequently testing the animal for the existence of a conditioned reflex for these two gongs, it is possible to tell whether the animal has been able to distinguish between their pitches or not. Thus, if both of them are accompanied by secretion of the same number of drops of saliva on the average, then quite clearly they have sounded alike to the dog. By first testing with gongs which are very different in pitch, but in subsequent experiments using gongs which are closer and still more close, it is possible to ascertain precisely at what point discrimination ceases. Since very great care has to be taken to see that it is really nothing but the pitch which is the criterion that determines the dog's behaviour, tuning forks or organ pipes emitting pure tones will be preferable to gongs, and it would be necessary to be sure that no other quality changed at the same time, which would enable the animal to differentiate between them. Thus, one organ pipe might emit a slight high-pitched hiss when it was

sounded. This might be quite inaudible to us but easily discerned by the dog. We should think that the dog was discriminating between the tones by their pitches, while in point of fact it was discriminating by the hiss in one of them.

REINFORCEMENT. Suppose that an animal in which a particular conditioned reflex has been established be not experimented with for several months, it will probably be found that the strength of the conditioned reflex has fallen somewhat from the value it had when the experiments ceased. This phenomenon is called decay. Experiment shows that a very few applications of the conditioning factors suffice to restore the reflex to its original value. This phenomenon is called reinforcement.

LINKING. Suppose that an animal with a conditioned reflex established between salivation and the sounding of a gong is experimented with for a few days by sounding a gong and a whistle at the same time. Then on subsequently testing the animal with the whistle it will be found that the conditioned reflex has a strength far greater than could be accounted for by the few times that the sound of the whistle and the feeding have been associated. What apparently has happened is that the conditioned reflex established for one sound has in great measure been transferred to the second. This phenomenon is called linking.

EXTINCTION. Suppose a conditioned reflex to have been established between the salivary reflex and the blowing of a whistle. Then, if for a number of times in succession the whistle be blown but no food given, it will be found that the strength of the conditioned reflex quickly dies away. This phenomenon is called extinction.

DELAYED AND TRACE REFLEXES. It is clear that the fundamental experiments with the modifications just described can be elaborated in a number of different directions. One of these consists in varying the time that is allowed to elapse between the application of the conditioned stimulus and the subsequent unconditioned stimulus. For example, the sounding of a note and the giving of food may occur simultaneously, or any number of minutes may be allowed to elapse between the sounding of the note and the giving of the food. If they occur together the conditioned reflex is called *simultaneous*. If a short time is allowed to elapse between the two, they are called *delayed*, and if the time is longer they are called *trace reflexes*. Now, as one would expect, delayed reflexes take longer to establish and are more liable to fade than simultaneous reflexes, and the same is still more true of trace reflexes.

Now it is clearly possible to vary in the opposite direction the time relations between the sounding of the gong and the giving of the food, namely, to give the food first and then to sound the gong. Experiment shows that unless the time be very short the establishment of a conditioned reflex becomes difficult or impossible. This is because the animal, as the result of repeated associations of the sounding of the gong and the giving of food, forms an association between the two, that is to say, the mechanism which sounds the gong is in some way connected with that which produces the food. If, on the contrary, the food is given first, so far as the animal is concerned subsequent events happening to the gong are of little or no interest.

INHIBITION. EXTERNAL INHIBITION. Suppose that between the time of sounding the gong and the giving of food an additional unexpected stimulus be introduced, such, for example, as playing a gramophone record. Then it is found that this extra stimulus has a marked depressant effect on the strength of the conditioned reflex. The first application of this

unexpected stimulus has a more powerful depressing effect than subsequent ones, as the following table shows :—

Strength of visual conditioned reflex	. . . . =	100 per cent.
Effect of gramophone, 1st application	. . . . =	10 ,,
,, ,, 2nd ,,	. . . . =	50 ,,
,, ,, 3rd ,,	. . . . =	65 ,,
,, ,, 4th ,,	. . . . =	85 ,,
,, ,, 5th ,,	. . . . =	90 ,,
,, ,, 6th ,,	. . . . =	94 ,,
,, ,, 7th ,,	. . . . =	100 ,,

This effect is known as external inhibition. Experiments of this nature make it clear that considerable pains have to be taken to eliminate the entry of unintentional external inhibition when initiating or testing the strength of conditioned reflexes. It has been found necessary to design special rooms (Fig. 220) provided with automatic means of giving the animal food at the

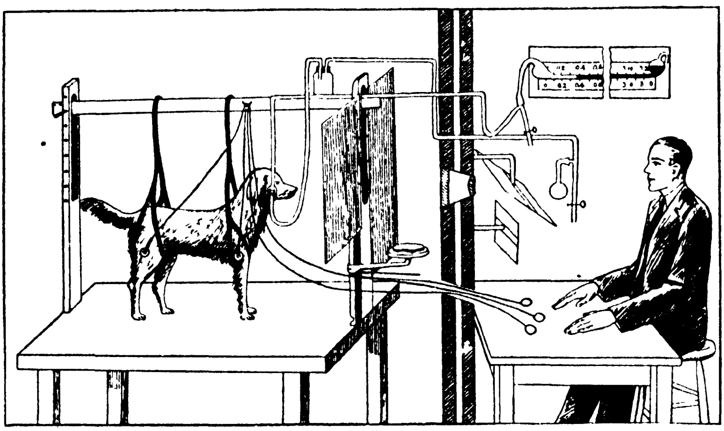

FIG. 220. Operator with various controls outside the cell observing animal inside the chamber by periscope. (PAVLOV.)

required instant of time and for supplying the various forms of stimulation, gongs, whistles, &c., &c., so that adventitious stimuli of an unwanted kind are avoided so far as possible.

INTERNAL INHIBITION. Besides the type of inhibition described in the previous section, other varieties of inhibition are met with, some of which can be classed as internal inhibitions. For example, as the result of careful training a conditioned reflex may be set up for an organ pipe producing a note of 256 vibrations per second, whereas another similar pipe, producing a note of 244, is ineffective. In this case discrimination of the pitches of the two pipes has enabled the animal to react correctly to one and to inhibit the effects of the other. This is one type of internal inhibition.

EFFECT OF EXTERNAL STIMULI ON INTERNAL INHIBITION. We have already seen that adding an extra stimulus of an unexpected nature immediately before or after a stimulus to which a conditioned reflex has been set up may result in marked inhibition in the strength of the conditioned reflex. We must now consider the effects of putting an additional unexpected stimulus just in front of a stimulus to which internal inhibition has been established. For example, having caused the animal to discriminate between a tone of 256 to which he salivates, and another of 244 to which he does not, let us investigate what will happen if, just prior to sounding the pipe of 244,

an unexpected additional stimulus is given to the animal. The result is that the animal salivates as if it was responding to the tone of 256 vibrations per second, and this clearly indicates that the internal inhibition may be temporarily removed by this unexpected extra stimulus.

SEAT OF INHIBITION. We must now consider whether the external inhibition which we have been considering develops primarily in the cortical area to which the normal stimulus goes, or whether it is associated more firmly with the cortical area to which the unexpected stimulus travels. Several observations seem to suggest that the inhibition arises within the cortical area to which the end organ concerned in the conditioned reflex connects. Suppose, for example, that as the result of repeatedly stimulating an area of skin and giving food, the conditioned reflex has been set up, and that a loud noise has been used to inhibit the conditioned reflex. Then experiment seems to show that the inhibitory after-effect will be much more marked in the cortical area representing the skin than in the cortical area representing hearing. The following experiment makes this clear. Suppose that a conditioned reflex has been set up for a particular area of skin, and that this has been inhibited by a loud sound so that the stimulation of skin only means to the animal to 'expect food,' whereas the loud sound and the stimulation of skin together mean 'no food.' Now, during a subsequent period another skin stimulus is used at the same spot for establishing a new reflex, for example, the contraction of a particular muscle instead of the secretion of saliva. Then, for the first time that the loud sound is applied at the same time as stimulation of the skin, there will be found to be inhibition of the movement of the muscle, just as there was inhibition of the flow of saliva. This clearly indicates that the association is between areas of cortex and not between the final paths used for the reflexes in question.

CONDITIONED REFLEXES OF HIGHER ORDERS. It is clear that by using conditioned reflexes in various combinations, sometimes using the same stimulus for two different reflexes, sometimes using a stimulus which has been used to inhibit one reflex to bring about the operation of a second one, we can link up conditioned reflexes in a very complicated way, only being limited by lack of time or of our own ingenuity. One example may be given. Suppose that a stimulus A be associated with the giving of food so that a conditioned reflex has been set up. Suppose that another stimulus B has been used to inhibit it, so that A and B together signify that no food is coming. Suppose now that for several times in succession the inhibitory stimulus B is associated with a third stimulus C which the animal has not previously experienced. Then on trying A and C together it will be found that C inhibits just as effectively as B did.

REMOVAL OF OR DAMAGE TO CORTICAL AREAS. Dogs which have survived the experimental removal of their cerebral cortex suffer a complete loss of the ability to form conditioned reflexes. Local damage only causes a temporary effect on conditioned reflexes if the damaged areas are of small size. Further, the damage appears to affect inhibitory stimuli more than excitatory ones. If, on the other hand, extirpation of the whole of the cortical area corresponding to a particular sense organ has been carried out, then many, but not all, the effects previously produced by stimulating this sense organ are lost. Such conditioned responses as are retained are probably effected by sub-cortical mechanisms.*

HEREDITY OF CONDITIONED REFLEXES. One experiment to test this was performed as follows: Five puppies were removed from their mother and were fed entirely on milk. The first puppy had a conditioned

* MARQUIS and HILGARD. *J. Comp. Psychol.*, 1936, **2**, 157.

reflex established between the giving of food and the sound of a metronome; the second puppy to the stimulation of the skin by an induction coil; the third to the smell of camphor; the fourth to the appearance of a red light; and the fifth to a white light, which periodically went on and off. The conditioned reflexes developed quickly, and on testing were found to be quite stable. When the puppies were several months old they were shown a number of different objects, amongst them being pieces of meat and other foods which their ancestors had been acquainted with. No salivary activity was observed in the puppies. It did not matter whether the animals were merely shown the objects in question or were allowed also to smell them. The results were the same. Meat and rubber corks alike were indifferent to them. We see, then, that the salivary reflex to sight or smell, which might be supposed to be a hereditary characteristic possessed by all dogs, is in point of fact an acquired conditioned reflex.

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SLEEP

All animals have, during each day, one or more periods of rest in which there is a general reduction in the activity of the central nervous system, but especially of the cerebral cortex The reduced, or delta, electric waves in the cortex are evidence of this. Adult human beings normally have one such sleep period in the twenty-four hours.

The need for, or onset of, sleep is announced by subjective feelings of drowsiness, dryness of the conjunctivæ, difficulty in keeping the eyes open, and general muscular relaxation.* When sleep sets in, the muscular relaxation increases, and before awakening, tonus returns. Many methods have been used for recording the movements made during sleep; the closing of electric contacts by movement of the bed springs, the use of smoked drum and lever, or cinema photography of the sleeper. Kleitman, Cooperman and Mullin † used a combination of some of the above methods with a machine which added together the vertical displacements of the centre of the bed springs. They also recorded the rectal temperature. Although most people make some movement every few minutes, little time is occupied by movements during sleeping, usually less than half a minute per hour. More time is spent in movement towards morning. There was no observable connection between rectal temperature and movement.

During sleep the knee jerk disappears, but various superficial reflexes remain, and a positive Babinski sign is always obtained on stroking the sole of the foot. Urine secretion is reduced, and sometimes also gastric secretion and movement, but hunger contractions of the empty stomach may be increased. Vasomotor reflexes are conspicuous. The eyes are usually directed upwards, but any position may be taken up by either eye. The pupils are contracted, but contract still further to light. The heart rate is slowed, largely because of the general inactivity of the body, and the arterial blood pressure is lowered about 10 to 20 mm.‡ There is some dilution of the plasma and increase of blood volume.§ Basal metabolic rate is reduced, but the respiration rate may be lowered, raised or unchanged.

* KLEITMAN. *Amer. J. Physiol*, 1923, **66**, 67.
† KLEITMAN, COOPERMAN and MULLIN. *Amer. J. Physiol.*, 1933, **105**, 574.
‡ LANDIS. *Amer. J. Physiol.*, 1925, **73**, 551.
§ COOPERMAN. *Amer. J. Physiol.*, 1936, **116**, 531.

The depth of sleep may be measured in a number of ways. One way is to take a record of the blood pressure, and to ascertain by experiment how loud a sound is necessary to cause a noticeable change in this. It is found that there are wide individual variations, but often sleep increases in depth until, two or three hours after the onset of sleep, it reaches its maximum. After this it decreases until about six hours after the onset of sleep, and then stays approximately uniform. Just before the time to wake up is reached, it becomes very light. Deep sleep is dreamless, and when dreaming does occur it is probably the result of some afferent impressions ; these are not subjected to the normal critical examination in the cortex, and so are usually grossly misinterpreted.

Prolonged sleeplessness in man leads to no very conspicuous objective changes, except as regards the results of certain psychological tests. Thus arithmetical problems are less accurately solved, and it is generally agreed that the driving of a motor car would be less reliable. In later stages it becomes difficult to stand erect, and hallucinations may occur. In order to keep awake after a certain time continuous muscular activity is essential. When very prolonged, sleeplessness leads to collapse and death. In such cases chromatolysis and shrinkage of cells in the frontal areas of the cortex are found.

As the result of experimental work on sleep, several hypotheses have been advanced to explain it. One of these, due to Pavlov, ascribes the phenomenon to a spread of internal inhibitory processes. If, for example, an animal has been trained to expect food half an hour after an auditory stimulus has been given (a trace reflex) and that this time interval be made longer and longer, the animal tends to go to sleep during the intervening period. When the time interval of the experiment is over the animal wakes up and exhibits the normal salivary reflex.

Kleitman regards sleep as a reversible inactivity of the highest cortical centres, which is due to a functional break between it and the lower parts. The highest levels are least organised and most sensible to fatigue. The break is due to a reduction in the number of afferent impressions, especially in the proprioceptive ones dependent on muscular tone. Hence muscular relaxation, whether voluntary or due to fatigue, is a necessary condition for it. This view receives important confirmation in the experiments of Bremer, who found that when nearly all afferent impressions were removed from the cerebral cortex in cats by section of the mid-brain, the head showed all signs, including the slow electric waves, of deep sleep. The diurnal alternation of wakefulness and sleep is probably a conditioned phenomenon.

Other theories presuppose that some special centre is concerned in the production of sleep. Hess * was able to induce immediate sleep at any time in cats by stimulation of an area lying between the subthalamus and the thalamus, and near to the bundle of Vicq d'Azyr. The mechanism of this undoubted fact is unexplained, and it is difficult to suppose that sleep can be fundamentally an excitatory phenomenon. A 'sleep centre' was postulated by v. Economo,† but it would be more appropriate to assume a 'waking centre' the depression of which would cause sleep.

Ranson ‡ has indeed found evidence for such a 'centre.' He produced sleep. and sometimes catalepsy, in monkeys by bilateral lesions in the lateral hypothalamic area near the mammillary bodies, and similar results have been reported as a consequence of hypothalamic lesions in

* HESS. *Arch. f. Psych.*, 1936, **104**, 548 , *Lancet*, 1932, **223**, 1259.
† v. ECONOMO. *J. Neur. & Ment. Dis* , 1930, **71**, 249,
‡ RANSON. *Arch. Neurol. & Psych.*, 1939, **41**, 1.

man. This is explained as due to the loss of the emotional drive which normally emanates from the hypothalamus, so that a stage of quiet and relaxation results. It is the converse of the state in the decorticate animal (p. 248) where the hypothalamic activity is uncontrolled and leads to extreme excitability and 'sham rage.' It was also found by Demole * that injections of 2 mg. of calcium chloride into the region of the tuber cinereum caused sleep lasting several hours. It could be reversed by injection of KCl. In pathological conditions of hypersomnia any lesions present are usually in the region lying between the corpora mammillaria and the third nerve nucleus.

Since the volume of the brain increases in sleep † there can be no possibility that sleep is primarily due to cerebral anæmia, as often supposed.

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* DEMOLE *Arch f exp Path.*, 1926, **120**, 229
† SHEPARD. "The Circulation and Sleep." New York, 1914.

CHAPTER XXII

THE CEREBELLUM

MORPHOLOGY

MANY attempts * have been made to systematize the anatomy of the cerebellum, with the result that the terminology is at present in a chaotic state. The cerebellum consists of a central part or vermis, and two lateral hemispheres. The vermis on the upper aspect is called the superior vermis

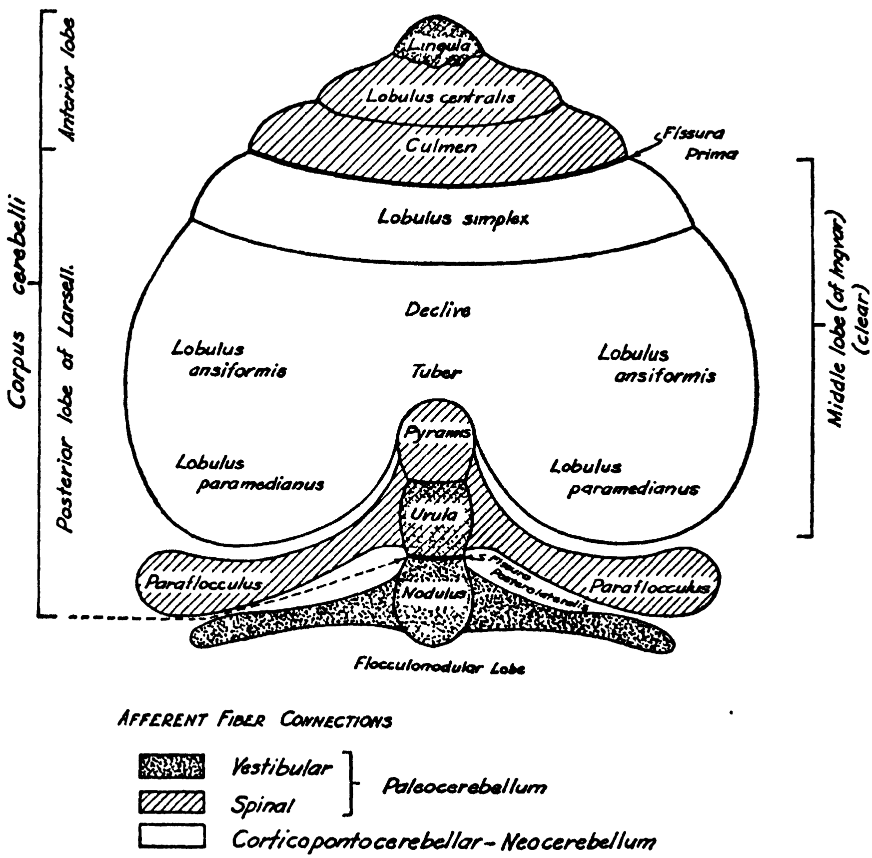

FIG. 221. Diagram of the Cerebellar Cortex of the Macaque, after Larsell. The surface is supposed to be opened out and spread in one plane. The principal divisions and afferent fibre connections are shown. The fissura posterolateralis separates the cerebellum into the small flocculo-nodular lobe and the larger corpus cerebelli. (FULTON. "Physiology of the Nervous System." Oxford University Press, 1938.)

* BOLK. "Das Zerebellum der Säugetiere" (1906) (Jena. Fischer). ELLIOT SMITH. *Anat. Anz.*, 1903, **23**, 368. INGVAR. *Folia Neurobiol.*, 1918, **9**, 205.

and that on the inferior aspect the inferior vermis (nodule to tuber in old terminology). According to Larsell,* whose subdivisions we shall use, the cerebellum consists of two main divisions :—(1) a smaller *flocculo-nodular lobe,* consisting of the flocculi, the nodulus and their peduncles, and (2) a larger *corpus cerebelli,* the two being separated by a fissure (not very conspicuous in man), the postero-lateral fissure.

The corpus cerebelli is again subdivided by the fissura prima, which runs transversely, into an *anterior lobe* and a *posterior lobe*, each further subdivided into smaller lobules by smaller fissures. Other folds run in such a way as to seem to divide the organ into a number of transverse laminæ. Ingvar recognizes a middle and a posterior lobe in the corpus, but Larsell considers both as posterior lobe (Fig. 221).

The anterior lobe, the posterior part of the posterior lobe, and the flocculo-nodular lobe are the phylogenetically old parts, and hence may be called the *palæocerebellum.* They represent the original cerebellum as a structure developed chiefly in connection with the vestibular centres of the medulla. Their fibre connections as shown in Fig. 221 are vestibular and spinal. The anterior, and larger, part of the posterior lobe (= Ingvar's middle lobe) is greatly developed in higher animals and especially in primates: it is the *neo-cerebellum* and is chiefly connected, *viâ* the pons, with the efferent tracts from the cerebral cortex. The cerebellum connects with the rest of the central nervous system by means of three pairs of peduncles: the *superior peduncles* or brachia conjunctiva, the *middle peduncles,* which form the pons. and the *inferior peduncles* or restiform bodies. The cerebellum is covered with a grey cortex, within which is white matter.

THE CEREBELLAR NUCLEI are the three pairs of roof nuclei, viz., the *Nn.fastigii, globosus,* and *emboliformis* and the lateral *nucleus dentatus,* the last-named being the largest. Part of the dentate nucleus is connected with the palæocerebellum and part with the neo-cerebellum. All the nuclei are placed deeply and separated from the cerebellar cortex by the white matter. The general plan of connections is that the afferent fibres to the cerebellum go chiefly to its cortex: from this, fibres relay to the cerebellar nuclei, and from these again fibres pass out to other parts of the central nervous system, chiefly by the superior peduncles.

HISTOLOGY OF THE CEREBELLUM. The cerebellar cortex is thrown into laminæ or folia by the transverse arrangement of the smaller fissures. Its substance shows three layers, and their structure is identical over the whole cortex. Outermost is the molecular layer, next a layer of Purkinje cells, and on the inside a granular layer. Beneath this is the white matter.

Starting at the deepest layer, the *granular layer,* this consists of small 'granule' cells with short dendrons and long axons; the latter ascend to the molecular layer, where they bifurcate, the two branches then running parallel to the surface and parallel to the folia, *i.e.* transversely across the cerebellum (Fig. 222). Golgi type II cells are also found in the granular layer.

The *Purkinje cells,* of the next layer, have large bodies with freely branched dendrons that extend throughout the molecular layer and make synapses with the parallel axons of the granule cells (Fig. 223). The dendrons of the Purkinje cells are spread out in a plane which is at right angles to that of the folia. Their axons pass to the cerebellar nuclei.

The *molecular layer,* in addition to the ascending axons of the granular cells, and their horizontal branches, contains small stellate cells and the

* LARSELL. *Arch. Neurol. Psych.*, 1937, **38**, 580.

so-called *basket cells*, the dendrites of which run at right angles to the folia, *i.e.* sagitally. They send out collaterals which end by basket-like arborizations round the bodies of Purkinje cells, so that several of these can be affected by one basket cell.

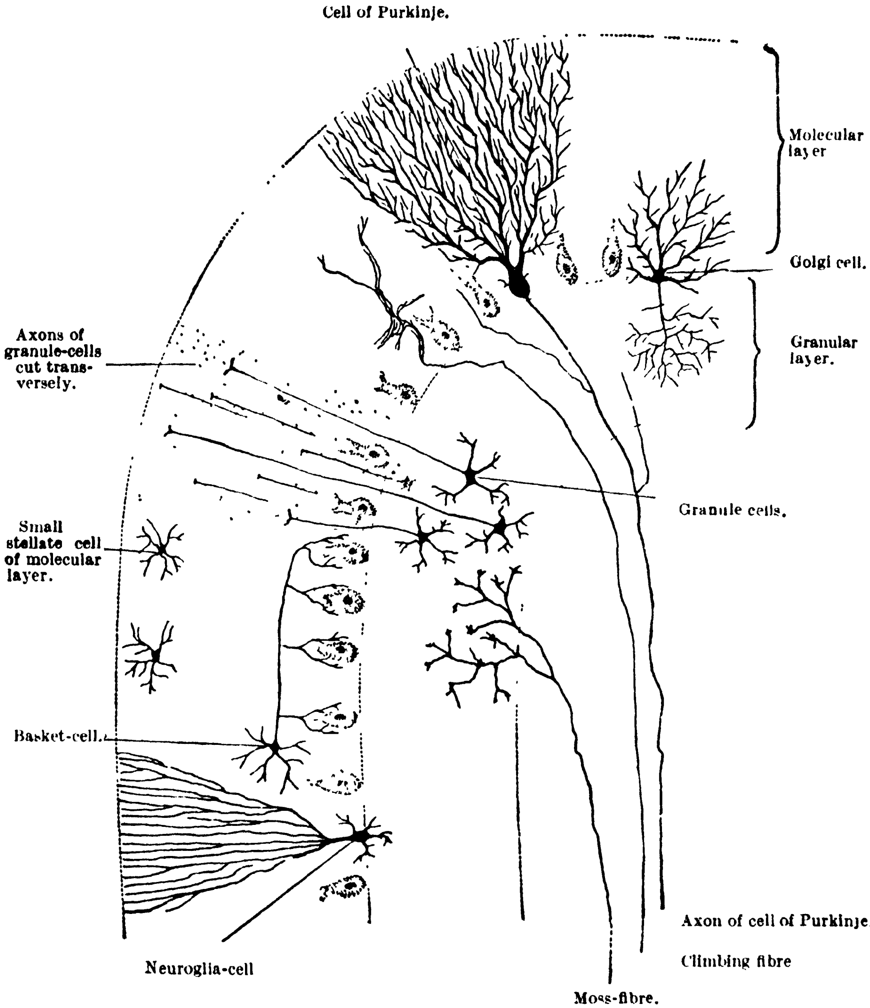

FIG. 222. Transverse Section of a Cerebellar Folium. (GRAY'S "Anatomy.")

The *afferent fibres* entering the cerebellum go mainly straight to the cortex and are of two types :—(1) the *moss fibres* which end in the granule layer, and (2) *climbing or tendril fibres* which connect only with the Purkinje cells or their dendrons.

The moss fibres are derived chiefly from the spino- and olivo-cerebellar fibres and it will be seen that the arrangement of cerebellar neurones is such as to diffuse impulses from these widely over the cerebellum in a transverse direction by means of the parallel fibres and also in a sagittal direction by the Purkinje cells and the basket cells by which these latter are associated.

The climbing fibres on the other hand, derived mainly from pontine and vestibular sources, carry impulses that are less widely diffused. From the

climbing fibres they reach the Purkinje cells and the axons of these lead straight down to the cerebellar nuclei. Since some collaterals from the axons of these Purkinje cells are recurrent, there are no doubt opportunities for self-re-exciting circuits by which discharges are reinforced and prolonged. Similarly, the Golgi cells provide further opportunities for the reinforcement of impulses (Fig. 223). The histological evidence then suggests that in the cerebellum incoming impulses are widely diffused and may also be augmented before their final emergence.

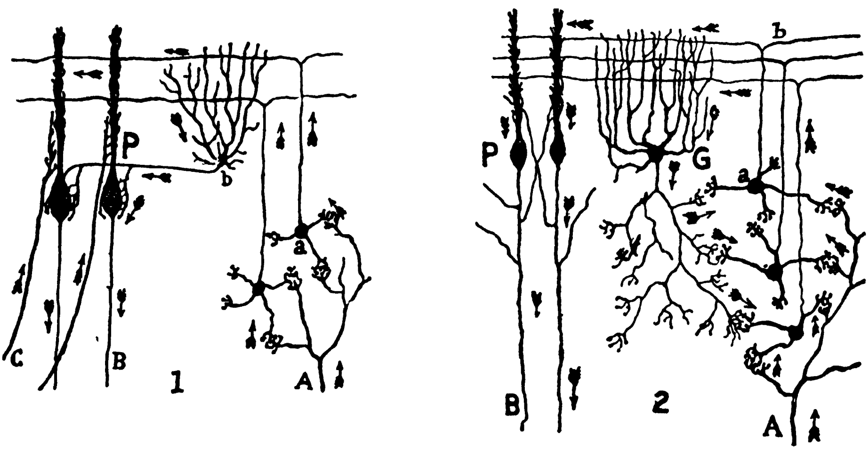

FIG. 223. Diagrams of Cajal showing two possible Paths for the Impulses carried by the Moss Fibres to the Cerebellar Cortex ; the arrows indicate the direction of transmission of the impulses. 1 The unidirectional arc, and 2, the recurrent arc through the Golgi cells (G) A, moss fibre ; B, axons of Purkinje cells, C, climbing fibres ; a, granule cells ; b, basket cells ; P, Purkinje cells (From LORENTE DE NÓ. *Arch Neurol. Psych.*, 1933, **30**, 245.)

CONNECTIONS OF THE CEREBELLUM

The cerebellum receives the major part of the proprioceptive impulses from all sources, and acts as an important centre for their co-ordination. Its efferent fibres make connections with the motor system at all levels of the central nervous system. If any clue to its functions may be discerned in these connections, it is that the afferent connections of the palæocerebellum (roughly speaking, the vermis in older nomenclature) are vestibular and spinal, while those of the neocerebellum (the lateral lobes) are with the cortico-spinal and cortico-pontine projections.

The following are among the most important of its numerous connections (Fig. 223A).

A. AFFERENT CONNECTIONS

By the inferior peduncle

(1) *Vestibulo-Cerebellar Tracts.* From the vestibular nerve, and nuclei *viâ* the inferior peduncles to

(*a*) the flocculo-nodular lobe and part of the vermis of the anterior and posterior lobes (Fig. 221).

(*b*) the roof nuclei (chiefly fastigii).

(2) *Arcuate fibres* arising from cells of the nuclei gracilis and cuneatus, enter the inferior peduncle, and pass probably to the palæocerebellar cortex (not very important).

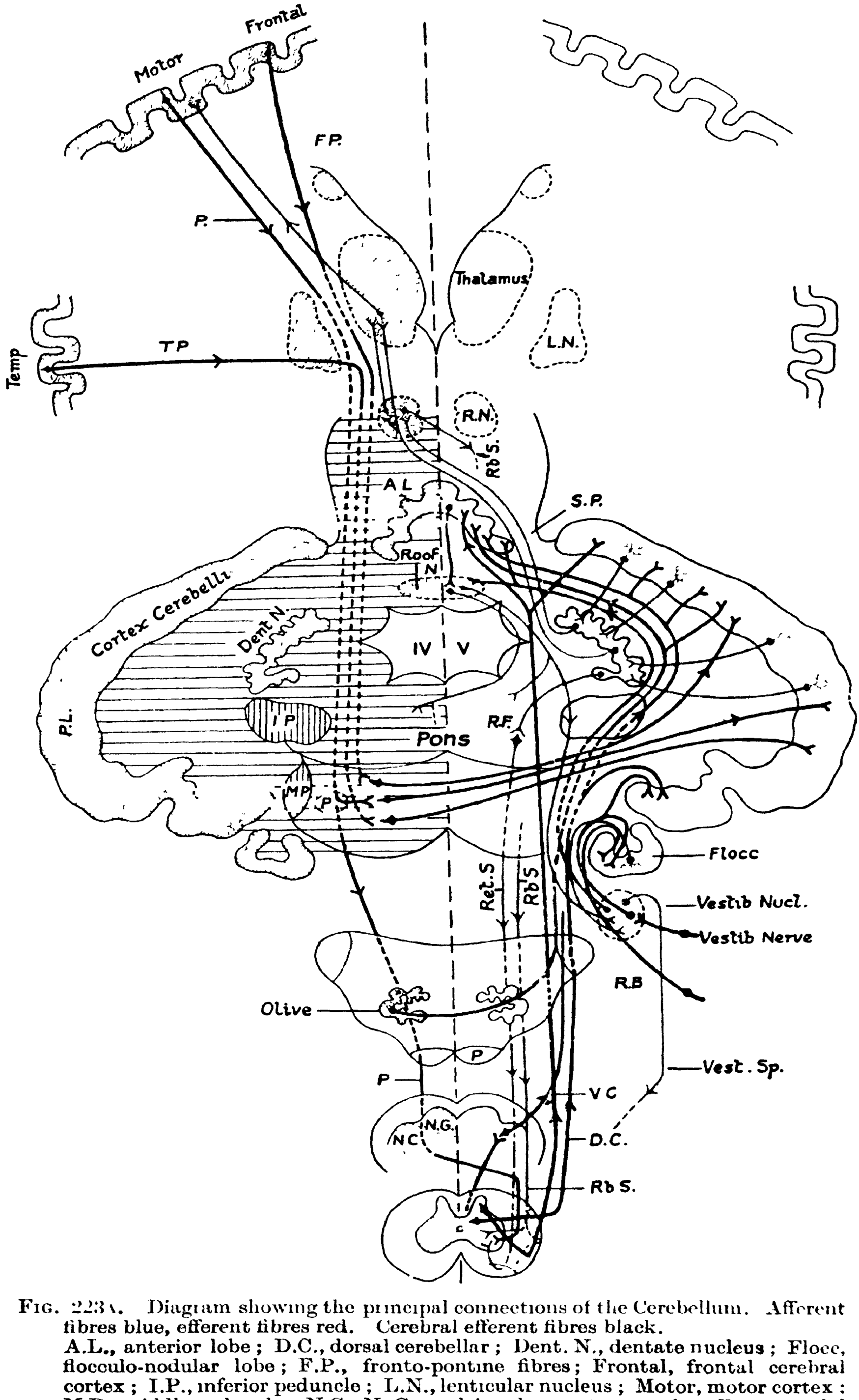

FIG. 223A. Diagram showing the principal connections of the Cerebellum. Afferent fibres blue, efferent fibres red. Cerebral efferent fibres black.

A.L., anterior lobe; D.C., dorsal cerebellar; Dent. N., dentate nucleus; Flocc, flocculo-nodular lobe; F.P., fronto-pontine fibres; Frontal, frontal cerebral cortex; I.P., inferior peduncle; L.N., lenticular nucleus; Motor, motor cortex: M.P., middle peduncle; N.C., N, G, nuclei and cuneatus gracilis; P, pyramidal fibres; P.L., posterior lobe; R.B., restiform body (blue fibres); Rb.S., rubro-spinal fibres; Ret. Sp., reticulo-spinal fibres; R.F., formatis reticularis. R.N., red nucleus; Roof. N., roof nuclei; S.P., superior peduncle; Temp, temporal cortex; T.P., temporo-pontine fibres; V.C., ventral cerebellar; Vestib. Nerve and Nucl., vestibular nerve and nucleus respectively; Vest. Sp., vestibulo spinal fibres; IV. V, fourth ventricle.

(3) *Olivo-cerebellar tract* fibres arise in the olive, cross over and pass up by the inferior peduncle to the cortex of the palæo- and neo-cerebellum.

(4) *Spino-cerebellar tracts.* (*a*) The *dorsal* spino-cerebellar tract fibres, with a contribution from the Vth cranial nerve, ascend by the inferior peduncle to reach the posterior parts of the anterior and posterior lobes of the same side.

By the superior peduncle

(*b*) The *ventral* spino-cerebellar tract passes up to the superior peduncle, and then turns back to enter the anterior lobe and roof nuclei of the same side.

By the middle peduncle

(5) *Cortico-cerebellar tracts.* Fibres from the cortico-pontine projections (= fronto-pontine and temporo-pontine) and branches from the cortico-spinal (pyramidal) tracts relay in cells of the *nuclei pontis*, axons from which conduct to the cortex of the neo-cerebellum of the opposite side.

B. EFFERENT CONNECTIONS. Fibres leaving the cerebellar cortex do so in two stages :—

I. *From cerebellar cortex to deep nuclei.*

II. *From deep nuclei* to extra-cerebellar structures.

The chief paths outwards fall into three groups :—

(1) *Cerebello-tegmental tracts* which lead from the deep nuclei to tegmental motor nuclei of the reticular formation in the brain stem :—

(*a*) Fibres from roof nuclei *viâ* inferior peduncle to vestibular nuclei,

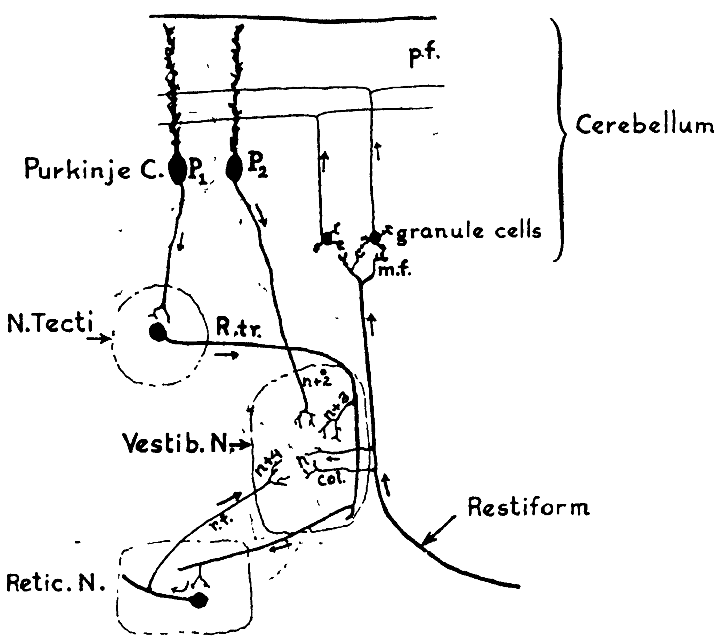

FIG. 224. Diagram of Pathways that Transmit the Proprioceptive Impulses carried by the Restiform Body to the Vestibular Nuclei. p.f., parallel fibres; m.f., moss fibre; R. tr. = Russell's tract, which gives off collaterals to the vestibular nuclei and ends in the reticular substance. From this, recurrent fibres pass back to the vestibular nuclei. (LORENTE DE NÓ. *Arch. Neurol. Psych.*, 1933, **30**, 245.)

posterior longitudinal bundle, reticular formation, reticulo-spinal tract, etc.
(*b*) Russell's tract from roof nuclei to vestibular nuclei, posterior longitudinal bundle, etc., of the opposite side (Fig. 224).
(*c*) From roof nuclei and dentate nucleus, (palæo-cerebellar part) by *superior peduncles*, fibres cross to the opposite side, and after sending collaterals to nuclei in medulla, pons and mid-brain, end in the large-celled part of the red nucleus. From these the rubro-spinal tract originates and this then crosses back again to the same side.

(2) *Cerebello-rubro-thalamic and cerebello-frontal tracts.* From the dentate nucleus (neo-cerebellar part) *viâ* the superior peduncles to the opposite side, and on to the small-celled red nucleus ; relays from here, together with direct fibres from cerebellar peduncles, pass on to relay in the thalamus, or pass directly to the frontal and temporal cerebral cortex.

The efferent tracts thus fall, like the afferent ones, into two systems, one putting the palæocerebellum into relation with the motor and correlation centres of the brain stem and spinal cord, and one putting the neo-cerebellum into relation with the cerebral cortex.

It is important to note that the cerebellum is connected with the spinal cord of the same side and with the cerebral cortex of the opposite side.

FUNCTIONS OF THE CEREBELLUM

Knowledge of the functions of the cerebellum is derived from experiments on extirpation and on stimulation, and from clinical observations of the effects of disease or injury.

EXTIRPATION. The effects of removal of the cerebellum differ according to the species of animal used and the extent of the ablation practised. It is necessary to distinguish between the effects of removal of the palæo-cerebellum and of the neo-cerebellum. The former affects equilibration and muscular tonus, the latter leads to impairment of voluntary movements.

In birds the whole cerebellum is a palæocerebellum, and when it is removed there follows a state of hypertonus of the extensor muscles.* Destruction of the anterior lobe alone (which has spinal connections) merely causes this extensor rigidity, but if the posterior lobe (with vestibular connections) is removed there is also defective equilibration, the head being bent backwards.

In mammals, removal of the whole cerebellum leaves sensation unaffected, modifies, though it does not prevent, voluntary movements, and causes disturbance of posture and equilibrium. There are great differences between species ; in lower mammals the effects are transitory, but in apes and men they may be long-lasting. Luciani † described three stages following cerebellar ablation in dogs :—(1) functional exaltation, (2) maximal cerebellar deficiency, (3) compensation.

In the first stage there is restlessness, with spasms of exaggerated tonus of the extensor muscles very similar to those of decerebrate rigidity. The positive supporting reaction of magnus is greatly enhanced.‡ The results are probably due to release from cerebellar inhibitory influences ; an increase

* LANGE. *Pflüger's Arch.*, 1891, **50**, 615 TEN CATE. *Arch Néerl. Physiol.*, 1926, **11**, 1 ; BREMER and LEY. *Arch intern. Physiol*, 1927, **28**, 58.

† LUCIANI. Il cervelletto, 1891 (Monnier, Florence). "Human Physiology," III, 1915, p. 419 (Macmillan, London).

‡ RADEMAKER. *Rev. neurologique*, 1930, **1**, 337.

of rigidity can in fact be demonstrated on removal of the cerebellum from a decerebrate animal.

In the second stage three symptoms appeared, called *asthenia* (weakness), *hypotonia*, and *astasia*—staggering gait and tremor on voluntary movement (intention tremor).

In the third stage these deficiency symptoms are in part remedied by the improvement of voluntary control. The animal walks with the legs wide apart and so obtains a broad and secure base.

Removal of half the cerebellum, on one side only, leads to the appearance of the characteristic symptoms on the same side of the body, but recovery is much more complete than after total ablation.

Equilibration is not profoundly affected by removal of the cerebellum, and the labyrinthine postural reflexes are unaffected by it if the vestibular nuclei are not damaged.

Destruction of different parts of the cerebellum leads to results which are in accordance with what we know of their connections. Thus, if the flocculo-nodular lobe is destroyed there are disturbances of equilibrium ; destruction of the anterior lobe causes increase of extensor tonus as in the pigeon, while ablation of the neo-cerebellum results in grave disturbances of voluntary movements with hypotonia and, if the dentate nucleus is involved, with tremor and errors in range of voluntary movement.

STIMULATION. Claims that precise muscular movements are obtainable by stimulation of the cerebellar cortex * have not received general approval, but slow movements of definite patterns have been got,† and the question cannot be regarded as finally settled. To produce movements stimuli must be strong, and the effects are probably due to spread of current to underlying structures ; at all events the effects are said not to be abolished by applying a solution of cocaine to, or by cooling, the cortex.

Weak stimulation of the cortex of the neocerebellum certainly produces no movements of the musculature, nor would it be expected to do so, in view of the fact that its projections are to the cerebral cortex and thalamus. It does, however, cause change in the motor areas of the cerebral cortex, as is shown by increase in its spontaneous electric rhythm ‡ and by an increase in its excitability.§

Stimulation of the anterior lobe does produce effects on the musculature, not contraction, but a relaxation of tonus, *e.g.* of that of decerebrate rigidity.‖ The relaxation is usually of the antigravity muscles of both limbs of the same side, with no contraction of the antagonists, and also a smaller effect, or more rarely an augmentation of extensor tone, on the opposite side. If a crossed extensor spinal reflex is set up beforehand it is inhibited. The relaxation is followed, after stimulation, by a rebound contraction.¶ Sometimes extensor movements are obtained, but there is no exact localization of these.

Stimulation of the flocculus causes movements and nystagmus of the eyes, which agrees with its known vestibular connection.

Stimulation of the deep nuclei ** leads to various movements which are interpreted as being changes of posture.

* MUSSEN. *Brain*, 1927, **50**, 313. *Arch. Neurol. Psych.*, 1930, **23**, 411.
† CLARK *J. Neurophysiol.*, 1939, **2**, 19.
‡ EARL WALKER. *J. Neurophysiol.*, 1938, **1**, 16.
§ BREMER. Le cervelet, Roger & Binet's *Traité*. Vol. **10**, p. 97.
‖ SHERRINGTON. *J. Physiol.*, 1898, **22**, 319.
¶ DENNY BROWN *et al.* *Proc. R. S.*, 1929, **104**B, 518.
** MILLER and LAUGHTON. *Arch. Neurol. Psych.*, 1928, **19**, 47 ; *Proc. R. S.*, 1928, **103**B, 575.

The effects of stimulation are abolished by section of the superior peduncles.

CEREBELLAR DISEASE AND INJURY

The effects of irritative lesions of the cerebellum are usually too much complicated by accompanying effects to enable definite conclusions to be drawn.

In destructive lesions of the cerebellum there seem to be two clear-cut conditions, attended with different symptoms, namely, destruction of the palæocerebellar parts, and destruction of the neocerebellum. In neither is sensation affected.

PALÆOCEREBELLAR DESTRUCTION. The palæocerebellum is insignificant in size in man, and hence is rarely affected alone. A midline tumour in children sometimes damages the flocculo-nodular lobe, and causes unsteadiness of gait, with walking on a wide base and a tendency to fall backwards. Tremor and hypotonia are not present, and voluntary movements of arms and legs are normal, though in walking there is inability to balance the body. A somewhat similar syndrome in adult men is seen in rare cases of primary palæocerebellar atrophy, due to degeneration of the Purkinje cells, chiefly in the anterior lobe. Here again the chief defect is in balancing the body on the legs; though voluntary movement of the legs is almost, and that of arms quite, unaffected. As in animals after removal of the anterior lobes, the proprioceptive reflexes concerned with equilibration are exaggerated, and the loss of equilibrium is no doubt due to this.

NEOCEREBELLAR DESTRUCTION. A definite syndrome results from loss of the lateral lobe or lobes of the cerebellum, many cases of which, resulting from bullet wounds were studied by Gordon Holmes. It is characterised by asthenia, definite hypotonia, disorders of voluntary movement, abnormal postures, and tremor on the same side as the lesion. These cause characteristic clinical signs, to many of which particular names have been given.

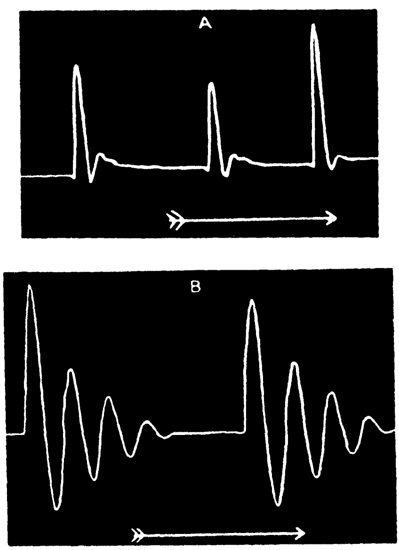

FIG. 225. Records of Knee Jerks. Normal jerk above. Jerk affected by cerebellar injury below. (GORDON HOLMES. *Brain*, 1918, **40**, 461.)

(1) *Hypotonia.* The muscles on the affected side are flabby, and although knee jerks are present, owing to depression of the normal plastic tone the jerk is of a lax and swinging character (Fig. 225). Hypotonia is probably the fundamental defect in neocerebellar lesions, and after removal of the whole cerebellum is also the dominant feature in some species of animals.

(2) *Disorders of Voluntary Movement.* Voluntary contractions are weak (*asthenia*), slow in onset and ending, and easily show fatigue (Fig. 226). They also show errors in

range and force (*dysmetria*). Hence rapidly alternating movements, such as pronation and supination, are difficult to perform (*adiadochokinesis*) (Fig. 227), and co-ordination

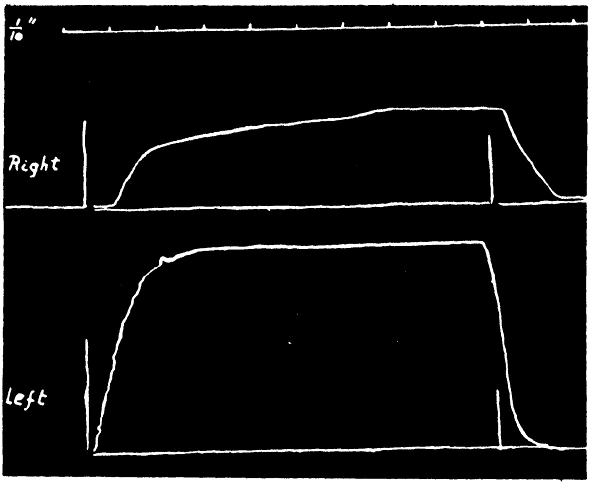

FIG. 226. Illustrating the Characters of Voluntary Contractions in Cerebellar Disease, which affected the Right Side of the Cerebellum. Slower and weaker contractions on the same side. (GORDON HOLMES. *Brain*, 1918, **40**, 461)

of movements is poor (*ataxia*), because of improper association of the agonist and antagonist muscles concerned (*asynergia*) In order to compensate for these disabilities the patient often performs a movement in stages, as though 'by numbers' (*decomposition of movement*). Speech disorders are sometimes present and are probably due to loss of delicacy and precision in muscular movements.

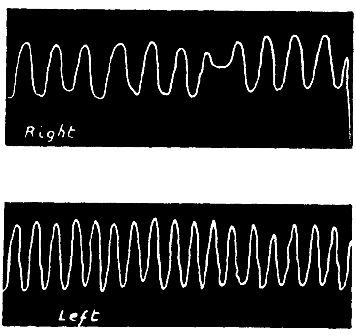

FIG. 227. Adiadochokinesis in Right-sided Cerebellar Lesion. Record of rapid supination and pronation movements of arm on injured side and on normal side (lower). (GORDON HOLMES. *Brain*, 1918 **40**, 461.)

(3) *Abnormal Postures.* Because of interference with proprioceptive impulses, abnormal attitudes of the arms or legs may result: thus the arm held forwards deviates outwards on the affected side if the eyes are closed (*Barany's pointing test*). There is also a tendency to fall or deviate towards the injured side; hence the gait zig-zags. Nystagmus is common, but vertigo rare.

(4) *Tremor*, of a coarse character, appears as soon as a muscle or limb begins to suffer fatigue, but is only conspicuous if the deep nuclei are damaged.

There is rapid compensation for the effects of the lesion, and it is on the whole remarkable how small the effect of a large cerebellar loss may finally be.

CONCLUSIONS REGARDING THE FUNCTIONS OF THE CEREBELLUM

Three points seem clear about the functions of the cerebellum. First, that it is in no way connected with sensation; second, that it has important relations to the maintenance of postural tonus; and third, that it is concerned

with the attainment of precision as regards force, timing and direction in voluntary muscular movements.

The phylogeny of the organ, its connections, and the effects of partial ablations all point to a difference of function as between the palæocerebellum and the neocerebellum. The former appears most clearly to be concerned in the fine adjustment and integration of the unconscious afferent impulses which play a part in the maintenance of the proper distribution of postural tonus. It is essentially proprioceptive, and related to the co-ordination of the reflexes of muscular tonus in its relation to equilibrium maintenance. It probably, among other actions, serves to moderate the proprioceptive reflexes concerned in the maintenance of equilibrium. In man this function is a subsidiary one.

It is perhaps not possible to draw a perfectly definite line separating off the functions of the neocerebellum from those of the palæocerebellum, because normal voluntary movements are played off on a background of postural tonus, but it is evident that the neocerebellum has important functions with respect to the execution of voluntary movements. We may suppose from the evidence that has been reviewed that it serves in some way to organize and, where necessary, to fortify the impulses from the cerebral cortex, and so to fit them into the existing background of postural tonus as to give precise, smooth and sufficiently powerful contractions of the various muscle groups in their correct relations to each other. Hence, in some way at present unknown, it is superadded to, and seems to exercise a kind of final editorial supervision over, the motor impulses emerging from the cerebral cortex. "By virtue of the proprioceptive impulses which it receives from all peripheral structures concerned in posture and movement, the cerebellum is in a position to send to the forebrain an integrated influx which may serve in the control and distribution of muscular tone" (Gordon Holmes, 1939). Local representation of muscular areas on its cortex is at most only vaguely indicated and indeed a study of its histological structure leads to the conclusion that its functions are generalized over its surface.

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CHAPTER XXIII

THE AUTONOMIC SYSTEM AND THE HYPOTHALAMUS

I. THE AUTONOMIC SYSTEM

THE autonomic nervous system includes those arrangements of efferent and afferent nerve fibres which are primarily concerned with the regulation of the internal processes of the body Its efferent fibres are distinguished from the somatic efferents supplying skeletal muscle by having on the course of each ultimate fibre one synaptic connection outside the central nervous system, viz in one of the various ganglia of the sympathetic and parasympathetic systems. Its afferent fibres are widespread and subserve many important reflex functions as well as being concerned in the appreciation of visceral pain.

The autonomic system has connections with, and tracts and centres in, the central nervous system proper, and according to the manner of the connections with the central nervous system, the efferent parts of the autonomic system are subdivided into two sub-systems of distinct, or even contrasting, function, viz. the *sympathetic* and *parasympathetic* systems.

We may therefore consider the autonomic system under these divisions :

(1) Sympathetic—or thoracico-lumbar } Efferent
(2) Parasympathetic— or cranio-sacral }
(3) Afferent autonomic fibres

(1) **THE SYMPATHETIC SYSTEM** (Fig 228) consists of the *lateral chain* of sympathetic ganglia, various *collateral ganglia* and plexuses, and many scattered *terminal ganglia*. The whole system originates from neurones limited to the intermedio-lateral cell columns of the thoracic and upper lumbar levels of the spinal cord, though the sympathetic chain extends from the upper cervical level to the coccyx. The cells of the sympathetic ganglia are all multipolar.

The sympathetic chain consists of bundles of nerve fibres, medullated and non-medullated, which connect together the '*lateral*' *ganglia* lying on each side of the vertebral column, there being as a rule one ganglion to each spinal nerve root. But in the cervical region these ganglia are condensed into two, the *superior and inferior cervical ganglia*, united by the cervical sympathetic trunk ; and the upper three or four thoracic ganglia on each side are condensed to form the '*stellate*' *ganglion*. At the bottom of the chain there is only one coccygeal ganglion for the coccygeal vertebræ.

The (lateral) ganglia of the sympathetic chain are connected with all the spinal nerves, just after they have given off their posterior divisions, by means of the *rami communicantes* These rami communicantes are of two kinds : *white rami* consisting of small medullated fibres, and *grey rami* composed almost exclusively of non-medullated nerves. It was shown by Gaskell that the white rami are formed by fibres which have their origin in the spinal cord and perhaps also in the posterior root ganglia ; whereas the grey rami

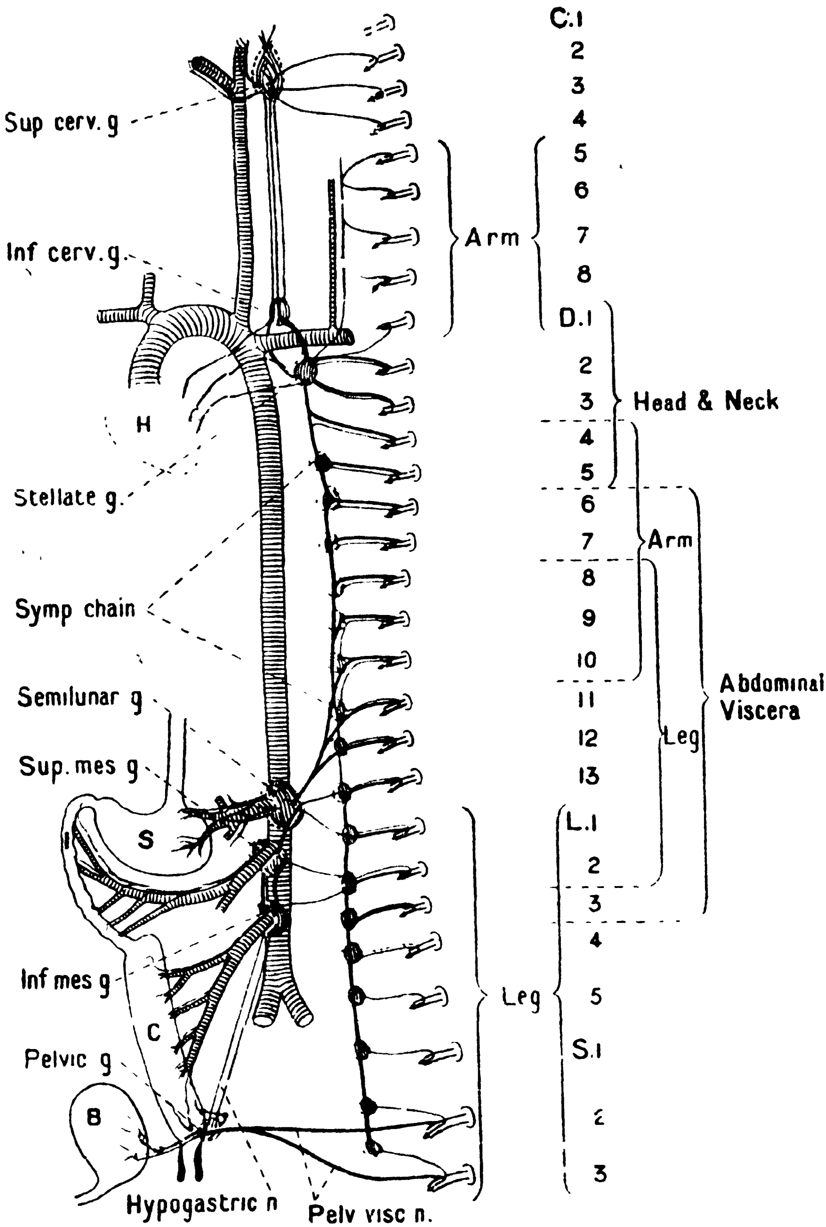

FIG. 228. Diagrammatic Representation of the Distribution of the Sympathetic and Sacral Autonomic Systems.

The black lines represent the medullated pre-ganglionic fibres, such as those making up the white rami communicantes, while the post-ganglionic fibres are printed in red. On the extreme right of the figure is indicated the general distribution of the white rami arising from the several nerve roots, while the double brackets point to the nerve roots making up the limb plexuses. (The sacral autonomic fibres arise from S2 and 3, and are parasympathetic.)

H. Heart.
S. Stomach.
I. Small intestine.
C. Colon.
B. Bladder.

represent fibres which, arising in the sympathetic ganglia, run back to join the spinal nerves (Fig. 229). The visceral outflow represented by the white rami is limited to a distinct region of the cord, viz. from the first thoracic to the third or fourth lumbar nerve roots; whereas the grey rami pass back from the sympathetic chain to all the spinal nerve roots. Stimulation of a limited number of white rami produces all the effects that can be evoked by stimulation of the grey rami, showing that the impulses leaving the cord pass upwards and downwards in the sympathetic system and are broken somewhere in their course, being transferred to a fresh relay which, by means of non-medullated nerves, carries them on to their destination.

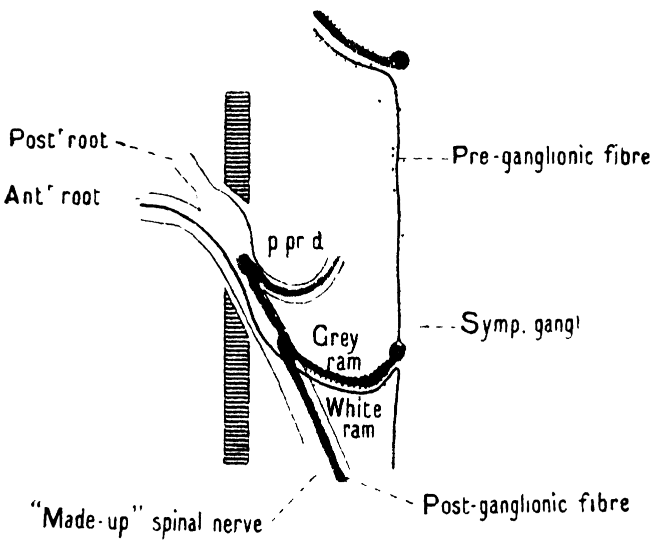

FIG. 229. Diagram to show the manner in which a Spinal Nerve is completed by the entry of a Grey Ramus, containing Fibres derived from Cells in the Sympathetic Chain. (After LANGLEY.)

p.pr.d. **Posterior primary division. (The post-ganglionic fibres are represented in red.)**

Every fibre of the sympathetic system at some point of its course forms one synapse with a nerve cell, and Langley has shown that this is the only cell-break in the fibre. This applies not only to the sympathetic fibres but also to the fibres of the other visceral nerves. Each fibre path therefore can be regarded as made up of two sections—a *pre-ganglionic* fibre arising in the spinal cord and passing out to a ganglion as a fine medullated nerve fibre, and a *post-ganglionic* fibre arising in this ganglion and continued as a non-medullated fibre, to its peripheral distribution.

This transference from one system to another involves the passage across a synapse and a nerve cell. The situation of this synapse may be easily revealed by utilising the action of nicotine, first studied by Langley.* If nicotine be applied to a sympathetic ganglion, it first stimulates and then paralyses any synapses which may lie in the ganglion. Intravenous injection of nicotine therefore causes a primary general excitation of all visceral ganglion cells. There is an enormous rise of blood pressure, which may be accompanied by other sympathetic effects, such as dilatation of the pupil, secretion of saliva, erection of the hairs, and so on. This rise rapidly passes off, and it is then found impossible to evoke any reflex visceral effects or

* LANGLEY. *J. Physiol.*, 1890, **11**, 123.

any contraction, *e.g.* of the blood vessels by stimulation of the spinal cord; the passage of the impulses is blocked in every one of the visceral ganglia. By observing the effects of stimulation of a nerve before it enters a ganglion and then painting the ganglion with nicotine, and again trying the effects of excitation, it is easy to determine whether the nerve fibres which were excited in the first case form any synapses with the nerve cells of that ganglion.

The relationships of the white and grey rami are strikingly illustrated in the case of the pilomotor fibres. These, in the cat, arise from the cord by the anterior roots from the fourth thoracic to the third lumbar inclusive. Passing by the white rami to the sympathetic system, they travel upwards and downwards and end by arborizations in the various ganglia of the main chain. From the cells of each ganglion a fresh relay of fibres starts, which runs as a bundle of non-medullated nerves (the grey ramus) to the corresponding spinal nerve, with which it is distributed to its peripheral destination. Each grey ramus causes erection of the hairs of one spinal segment, whereas stimulation of one white ramus causes erection in three or four segments, showing a distribution of the fibres of the white ramus to the cells in several successive ganglia. We thus see that, in speaking of the sympathetic functions of a spinal nerve root, we must clearly distinguish whether we mean the root as it arises from the spinal cord, in which case its visceral functions will include those of its white ramus, or whether we mean the made-up or complete spinal nerve after it has received its grey ramus (Fig. 229). In the latter case the visceral functions of the root will be more restricted than in the former case, and will have a different distribution.

The Collateral Ganglia. In the abdomen is a second system of ganglia, in special connection with the abdominal viscera, lying in front of the aorta and surrounding the origins of the large arteries to the alimentary canal.

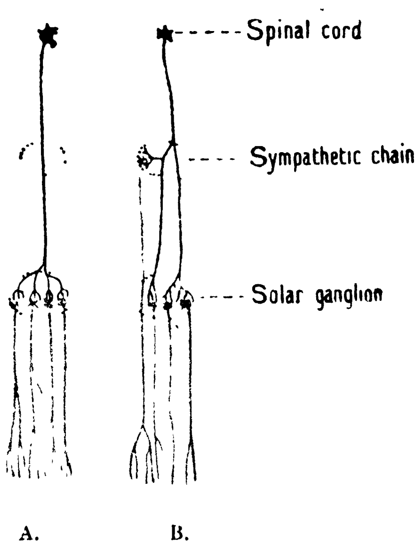

FIG. 230. Figure to show the probable Mode of Connection of the Fibres of the Splanchnic Nerve with Nerve Cells. (After LANGLEY.)

A. Usual type, all the fibres passing through the lateral chain to end in the collateral ganglia of the solar plexus.

B. Alternative condition, in which a small minority of the fibres have their cell stations in the sympathetic chain.

The pre-ganglionic fibres are black, the post-ganglionic red.

These are the semi-lunar, or cœliac, ganglion and the superior mesenteric and inferior mesenteric ganglia.

These ganglia are entangled in a fine feltwork of nerve fibres, which covers all the large arteries to form the sympathetic plexuses of the abdomen, *e.g.* the cœliac plexus, the mesenteric plexuses, hypogastric plexuses, &c. The outflow to these ganglia consists mainly of pre-ganglionic fibres, which have passed through the sympathetic chain without forming any synaptic connections there ; they emerge from that chain as *the splanchnic nerves.* With them are also a few post-ganglionic fibres (Fig. 230).

The three splanchnic nerves originate from the fourth to the eleventh thoracic spinal roots, and pass to the cœliac and superior mesenteric ganglia. Small branches from the lumbar roots pass in a similar way to both the mesenteric ganglia. The majority of the splanchnic fibres form their synaptic connections in the collateral ganglia (Fig. 230). From the abdominal plexuses, which are largely non-medullated, fibres pass to all the abdominal viscera. There are also some fibres of pre-ganglionic type which pass directly to the viscera, where they form synapses with ganglion cells in their walls.

DISTRIBUTION OF THE SYMPATHETIC FIBRES. The post-ganglionic fibres of the sympathetic system are distributed to their destinations in one of two main ways, viz. : with the spinal nerves, or by travelling along with the small arteries. The fibres of the grey rami are of the first type, the remainder are of the second.

There are grey rami to all the spinal nerve roots : this means that the grey rami may contain fibres derived from white rami at other levels, since the white rami are confined to the thoracic and upper lumbar roots. The pre-ganglionic fibre of the white ramus passes to the sympathetic chain and may travel up or down in it for one or more segments, giving off branches on the way, but ultimately it ends by synapse with many cells in one or more of the lateral ganglia. An impulse emerging from the cord by a pre-ganglionic fibre has therefore ample opportunity to affect many neurones of the chain, and to be dispersed along many differently distributed post-ganglionic fibres. Reflex responses of the sympathetic system therefore involve a wide and diffuse field of effectors.

(1) The ' post-ganglionic ' axons of the cells of the lateral ganglia enter the grey rami (often after some further travel up or down in the chain), and are then distributed to the region supplied by that particular spinal nerve root ; these areas are the skin of the limbs and trunk, the structures supplied being the arterioles and capillaries (vaso-constrictor), the sweat glands (secretory), the hair muscles or *arrectores pilorum* (motor, causing erection of hairs) : fibres also pass to the blood vessels of muscle, to which they are mainly vasodilator. The fibres for the *upper limb* leave the cord by the white rami of the upper thoracic roots, and have cell stations in the stellate and inferior cervical ganglia. Those for the *lower limb* are derived from the lower thoracic and upper lumbar roots. Those for the trunk areas come from the middle regions of the outflow from the cord.

(2) The remaining fibres of the sympathetic system are distributed by passing along the walls of the arteries, and most of the fibres to the skin of the head and neck are also of this type.

(i.) *The Head and Neck.* These fibres arise from the first and second thoracic nerves, ascend in the cervical sympathetic and have cell stations in the superior cervical ganglion, from which postganglionic fibres travel with the blood vessels, to supply all the structures of the head and neck. (*a*) The fibres to the *skin* supply sweat glands,

arrectores pilorum muscles, and small blood vessels. (*b*) The *salivary*, lachrymal, and buccal glands receive fibres that are secretory and vaso-constrictor. (*c*) The *brain* receives vaso-constrictor fibres. (*d*) The *eye* receives motor fibres to the *dilator pupillæ* muscle, to the *nictitating membrane* (when present), to the plain muscle of the orbit (*Müller's muscle*), and to some plain muscle in the upper lid. Stimulation of the upper end of the cut cervical sympathetic therefore causes dilatation of the pupil, retraction of the nictitating membrane and upper lid, and some protrusion of the eyeball, while section, or excision of the superior cervical ganglion, gives the opposite effects. (*e*) The *thyroid* also receives fibres, the function of which is uncertain.

(ii.) *The thoracic organs* are supplied from the second to fourth thoracic nerves, with their cell stations situated in the stellate ganglion, and, in man, also in the inferior and some even in the superior cervical ganglia. Post-ganglionic fibres are distributed with the cardiac, pulmonary and other plexuses. Those to the *heart* are accelerator and also dilate the coronary vessels: those to the *lungs* are broncho-constrictor and vaso-constrictor.

(iii.) *The abdominal organs* derive their pre-ganglionic fibres from the thoracic roots below the fourth or fifth, the fibres passing through the sympathetic chain to form the *splanchnic nerves*. These pass to the various collateral ganglia, the cœliac, and superior and inferior mesenteric ganglia and the pelvic or hypogastric ganglion, where the synapses are situated. Post-ganglionic fibres from these ganglia then travel along the blood vessels. These fibres, together with some pre-ganglionic fibres and numerous scattered small ganglia, form elaborate plexuses along all the main arteries of the abdomen, and are named after these arteries, *e.g.* renal plexus, &c The *hypogastric nerves*, derived from the lower levels of splanchnic outflow mainly relay in the hypogastric ganglion, the arrangement in man being different in detail from that in lower mammals.

All the abdominal and pelvic viscera are supplied with sympathetic fibres. (*a*) To the stomach and intestines, with the exception of their sphincters, the fibres are vaso-constrictor and inhibitory of movement. The sphincters are supplied with motor fibres, so that a result of sympathetic stimulation is a retention of contents in the alimentary canal. (*b*) The bladder is also supplied by inhibitory fibres, but its sphincter with motor fibres (*c*) The liver is supplied with fibres that constrict its blood vessels and also cause a discharge of glucose from the liver into the blood. (*d*) Weak contraction of the gall-bladder may also be caused by the sympathetic.

(*e*) To the spleen the sympathetic sends motor fibres to its plain muscle, and the organ diminishes considerably in size owing to expulsion of contained blood, when the sympathetic fibres of the splenic nerve are stimulated.

(*f*) Lastly, the splanchnic also sends an important branch to the suprarenal body, which discharges adrenaline into the blood stream when the splanchnics are stimulated. These *fibres that go to the suprarenal are pre-ganglionic fibres*, and instead of forming synapses with ganglion cells, they end *directly in relation to the cells of the suprarenal medulla*. It is interesting to note, however, that these cells are actually developed from the same embryological structures as form the sympathetic ganglion cells—a fact of some significance, as we shall see.

FUNCTIONS OF THE AUTONOMIC GANGLIA

These ganglia consist of a mass of nerve cells embedded in connective tissue, each cell being surrounded by a special capsule. The nerve cells are multipolar, each cell possessing one axon and several dendrites. The dendrites end in little arborizations round adjacent cells.

Since the main nervous system is characterized by the possession of nerve cells, it was formerly thought that any collections of nerve cells must partake of the co-ordinating and reflex functions of the central nervous system, *i.e.* must act as local nervous centres. Though theoretically conceivable, all efforts have failed to prove the existence of such a function, and we must conclude that the sole use of these ganglia is to serve as distributing centres. We may assume that one pre-ganglionic fibre divides, and the branches arborize round several cells (Fig. 231), whence new fibres arise to carry the impulse to the periphery—an impulse in the case of which there is no need for any minute localization. Indeed the essential part of a nerve centre is not the nerve cells at all, but the presence of a complex tangle of fibres, rendering possible the passage of impulses in all directions, the passage of an individual impulse being limited by reason of the varying resistance of the many possible tracts. Such a complex of fibres is found in mammals in the plexuses of Auerbach and Meissner, which do in fact act as local nerve centres for the intestine. But all such mechanism is wanting in the sympathetic ganglia

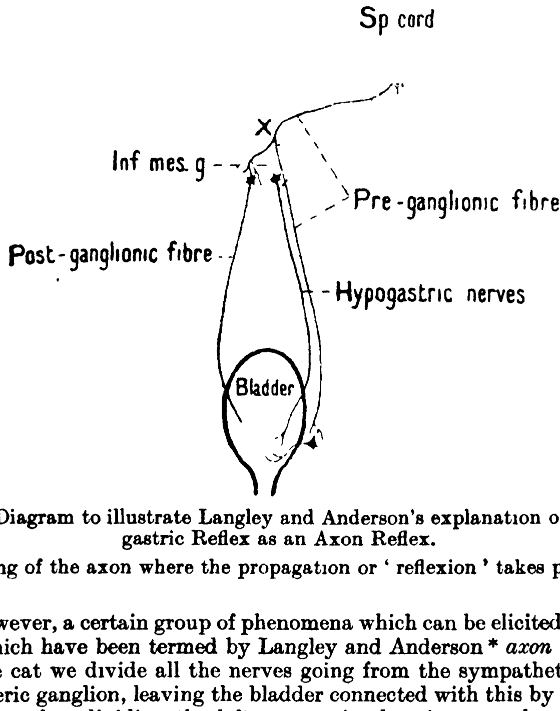

FIG. 231. Diagram to illustrate Langley and Anderson's explanation of the Hypogastric Reflex as an Axon Reflex.

The branching of the axon where the propagation or ' reflexion ' takes place is at X.

There is, however, a certain group of phenomena which can be elicited in sympathetic ganglia, and which have been termed by Langley and Anderson * *axon reflexes*. If, for instance, in the cat we divide all the nerves going from the sympathetic chain to the inferior mesenteric ganglion, leaving the bladder connected with this by the hypogastric nerves, and then after dividing the left nerve stimulate its central end, we obtain a contraction of the right half of the bladder. This effect is abolished by painting the inferior mesenteric ganglion with nicotine, showing that the activity of the cells of this ganglion is involved in the process. It has been shown, however, by Langley and Anderson that this is not a true reflex. A pre-ganglionic fibre arriving at the inferior mesenteric ganglion branches, one branch ending round the cells of the ganglion, while the other branch passes down in one hypogastric nerve to a cell situated near the base of the bladder (Fig. 231). When, therefore, we stimulate the central end of the divided nerve, we are stimulating some pre-ganglionic fibres, and the excitation spreads up to the point of junction of the two branches and then down the other branch to excite the cell in the inferior mesenteric ganglion. We thus obtain an apparent motor reflex by

* LANGLEY and ANDERSON. *J. Physiol.*, 1893, 16 410.

stimulation of a nerve which is itself motor. Similar pseudo-reflexes can be obtained for other viscera, *e.g.* the colon, *viâ* the hypogastric, as for the bladder, and also along the abdominal chain on the pilomotor nerves,* but furnish no grounds for ascribing to peripheral ganglia the property of reflex centres.

DISPENSABILITY OF THE SYMPATHETIC SYSTEM

Complete removal or inactivation of the sympathetic system has been performed on a number of animals by Cannon,† by removal of the thoracic and abdominal chains. Animals thus operated have lived for years in good health and of normal appearance, under the sheltered conditions of laboratory life. There is, however, a greatly reduced capacity to do work. This is because the body no longer has at its disposal the numerous reserves, such as heart acceleration, liberation of adrenaline, redistribution of blood to the working muscles, dilatation of bronchioles, &c., which are made available during exercise by the intermediation of the sympathetic nervous system. During emotional excitation or exposure to cold, also, there is none of the response associated with sympathetic activity, such as erection of hairs, or rise of blood sugar, or contraction of the spleen. It is therefore difficult to prevent a rapid fall of body temperature when the animals are exposed to moderate degrees of cold, in spite of the fact that the animals shiver much more readily than do normal animals under similar conditions. The same difficulty of regulating body temperature is seen when the surroundings are hot, so that dangerous rises of body temperature are likely to occur. It is clear, therefore, that the sympathetic nervous system, though not essential to the maintenance of life, when conditions are favourable, has as an important, if not as its chief function, the automatic adjustment of the manifold responses of the individual to the emergencies of an adverse environment.

(2) **THE PARASYMPATHETIC SYSTEM** consists of the craniosacral outflow of the autonomic system, and is divisible into the mid-brain, bulbar and sacral portions. Its pre-ganglionic fibres pass near to their final destinations before terminating in synapses, which, as shown by the nicotine method, are situated in peripherally placed ganglia.

The *mid-brain outflow* is represented by fibres in the *IIIrd* cranial nerve, which arises from the group of oculo-motor nuclei lying ventral to the aqueduct of Sylvius. The pre-ganglionic fibres pass to the ciliary ganglion in the orbit, from which they are relayed by post-ganglionic fibres to the *constrictor pupillæ muscle* and the *ciliary muscle*. Stimulation of the IIIrd nerve leads to constriction of the pupil, and contraction of the ciliary muscle. (Other fibres of the IIIrd nerve are not autonomic, viz. the nerves, both afferent and efferent, to the extrinsic eye muscles.)

The *bulbar outflow* comprises some of the fibres in the VIIth, IXth and Xth cranial nerves. In the *VIIth nerve* the fibres pass to the spheno-palatine ganglion, where relays of fibres occur for supply of the *lachrymal gland* and structures in the pharynx, and other fibres pass *viâ* the *chorda tympani* to peripheral ganglia for supply of the secretory and vasodilator fibres of the *submaxillary and sublingual* salivary glands. The *IXth nerve* similarly, *viâ* the otic ganglion, supplies the *parotid* gland and tongue with secretory and vasodilator fibres.

The *Xth cranial nerve* supplies most of the thoracic and abdominal viscera with parasympathetic fibres, which reach their destinations by way of various autonomic nerve plexuses, in which, as well as in the organs them-

* LANGLEY and ANDERSON. *J. Physiol.*, 1900, **25**, 364.

† CANNON, NEWTON, BRIGHT, MENKIN and MOORE. *Amer. J. Physiol.*, 1929, **89**, 84

selves, are placed the widely scattered ganglia on their course. The vagus fibres so distributed are inhibitory to the *heart*, vaso-constrictor to the heart vessels, constrictor to the *bronchi*, motor to the lower *œsophagus*, and augment movements of the *stomach* and *intestines*, with the exception of the sphincters, to which they are inhibitory. On the *stomach and pancreas* it has secretory functions, and also causes vaso-dilatation of the small blood vessels.

The *sacral outflow* leaves the spinal cord by the second and third sacral ventral roots, which form the *pelvic visceral nerves*, or *nervi erigentes*. These supply the pelvic viscera, the ganglia being mostly placed on their walls. The large intestine and rectum and bladder are supplied with motor fibres, and the external genitalia with vaso-dilator fibres. Stimulation of the pelvic visceral nerves causes erection of the penis as a result of the vaso-dilatation which follows.

COMPARISON BETWEEN SYMPATHETIC AND PARASYMPATHETIC

The various effects of the parasympathetic system are directed with greater precision of localization to their effector organs than is the case with the sympathetic system. With the latter, a pre-ganglionic fibre makes connection with several ganglion cells which are all situated at a distance from the effector organs or tissues, so that one fibre may produce an effect over a wide area. The parasympathetic fibres, on the other hand, proceed as pre-ganglionic fibres until they are near to their destinations, so that there is much less diffusion of their effect.

ACTION OF DRUGS. The widespread effects of the sympathetic system are also ensured by the fact that in states of sympathetic excitation, *adrenaline* is liberated from the suprarenal body into the blood, branches from the splanchnic nerves being secretory to the medulla of the suprarenal. The injection of adrenaline into the circulation reproduces most of the effects of sympathetic stimulation all over the body, *e.g.* dilatation of pupil, widespread vaso-constriction, liberation of glucose from the liver, &c. By circulating all over the body, therefore, the adrenaline which is set free by splanchnic stimulation, causes a generalization and prolongation of sympathetic effects in nearly all the organs that have a sympathetic supply. Thus, stimulation of the splanchnics not only causes relaxation of the tone of the stomach and intestines, but also acceleration of the heart and intense vaso-constriction of small vessels in the skin all over the body, even if the nerve supply to them has been cut..

Ergotoxine (ergotamine) paralyses the motor and secretory fibres of the sympathetic system, but has no effect on the inhibitory functions. If the drug is given, and the splanchnic then stimulated, or adrenaline injected, general vaso-dilatation occurs instead of constriction, so that the blood pressure falls. This is because there are sympathetic vaso-dilators, unaffected by the ergotoxine ; normally their action is masked by that of the more powerful vaso-constrictors.

Nicotine, as already stated, first excites and then paralyses all autonomic ganglion cells.

Cocaine greatly augments the effects of sympathetic stimulation or of adrenaline. *Ephedrine* has a similar effect, and it has been suggested by Gaddum that this is due to the fact that these substances protect the adrenaline from oxidation in the tissues.

Acetyl choline when injected into the blood or applied to a tissue reproduces all the effects of parasympathetic stimulation, and also causes secretion of

sweat, although the sweat glands receive a sympathetic supply. It also has a nicotine-like action in stimulating certain skeletal muscles and autonomic ganglia. Its action is very transient, however, as it is rapidly destroyed in the blood and tissues by the action of an enzyme called choline esterase.

Eserine exerts a toxic action on this enzyme, so that if eserine is injected into an animal, acetyl choline then causes intense and prolonged effects, *e.g.* slowing or inhibition of the heart, secretion of saliva, etc. After eserine the effects of parasympathetic stimulation are prolonged, and may also become generalized, *e.g.* stimulation of the vagus not only slows the heart, but also causes a secretion of saliva, etc.

Pilocarpine and muscarine also produce excitant effects on the structures supplied with parasympathetic fibres.

Atropine paralyses the actions of acetyl choline, and of pilocarpine; it also renders parasympathetic stimulation ineffective.

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THE CHEMICAL TRANSMISSION OF NERVOUS ACTION (THE HUMORAL THEORY)

The similarity between the effects of sympathetic stimulation and those of the administration of adrenaline have long been known, and have given rise to various explanatory speculations. More recently the similarity between the effects of stimulation of the various nerves belonging to the 'parasympathetic' system on the one hand, and the action of acetyl choline on the other hand, have excited attention; some instances are given on p. 210 and in Chapter XXXII.

As a generalization bringing these various facts into line, there has been propounded the 'humoral' theory, or theory of the chemical transmission of nervous action. According to this theory, the peripheral effects of sympathetic nerve stimulation are due to the release, *at the nerve endings* concerned, of an adrenaline-like substance, while those of 'parasympathetic' stimulation are due to the liberation of acetyl choline. The classical instance of humoral transmission of nervous action is that of the liberation of acetyl choline in the heart when the vagus is stimulated * (*v.* Chapter XXXII); the classical instances of sympathetic chemical transmitters have been provided by the researches of Cannon and his associates.† They found that when the splanchnic or the nerves to the liver were stimulated (the suprarenals having been previously removed), the heart of the animal (previously denervated) was accelerated, as it would have been had adrenaline been injected into the circulation. A similar substance was liberated when the sympathetic nerves to the cat's tail were stimulated, and was believed to have been formed in the smooth muscle of the *arrectores pilorum* muscles and blood vessels.

The action of the sympathetic substance, to which the name *sympathin* was given, like that of adrenaline, is augmented by administration of cocaine, yet it is believed that the substance is not necessarily adrenaline, since it differs from it in some of its physiological properties.

* LOEWI. *Pflüger's Arch.*, 1921, **189**, 239.
† *Amer. J. Physiol.*, 1931, **96**, 392; **97**, 365; 1932, **99**, 398.

Thus, sympathin produced by stimulation of the hepatic nerves resembles adrenaline in causing acceleration of the heart, contraction of limb and spleen volumes, and contraction of the nictitating membrane, yet, unlike adrenaline, it does not dilate the pupil or relax the uterus, and its action on blood pressure is not similarly reversed by the administration of ergotoxine. The sympathin obtained by stimulation of the cardiac sympathetic does relax the uterus.

In order to attempt to bring these facts into line, Cannon and Rosenblueth * postulate the formation, in the cells affected by the sympathetic, of two forms of sympathin, namely, sympathin E, which is formed when the action is an excitatory one, and sympathin I, formed when the effect is inhibitory; in addition there is a mediator, M (or adrenaline, A), which may travel in the blood. The local effect is produced by the combination of M (or A) with E or I, to form ME or MI. Different tissues produce different amount of sympathin E and sympathin I; thus the liver produces chiefly E, and the gastro-intestinal tract both E and I; the heart produces E from the cardiac muscle and I from the coronary vessels. Hence the varying effects produced on other organs when these substances get into the blood stream.

The 'parasympathetic' transmitter is believed to be identical with acetyl choline. This substance has been found in nervous and other tissues, and in the suprarenal gland; it has also been shown to be present in the blood coming from organs, such as the heart †, salivary glands, stomach, &c. when their parasympathetic supply is stimulated. Not only is it found in connection with the parasympathetic system, but has also been shown to be formed in the cells of sympathetic ganglia ‡ (and also in the medulla of the suprarenal, § which, morphologically, corresponds to a sympathetic ganglion), when these are thrown into action by nerve stimulation. Acetyl choline is thus the mediator, not only of the peripheral effects commonly called parasympathetic, but *also of the effects at all pre-ganglionic connections of the autonomic system.* For this reason it has been suggested by Dale that nerves which act by the production of acetyl choline at their terminations, *e.g.* the parasympathetic at their terminals, or the pre-gang-

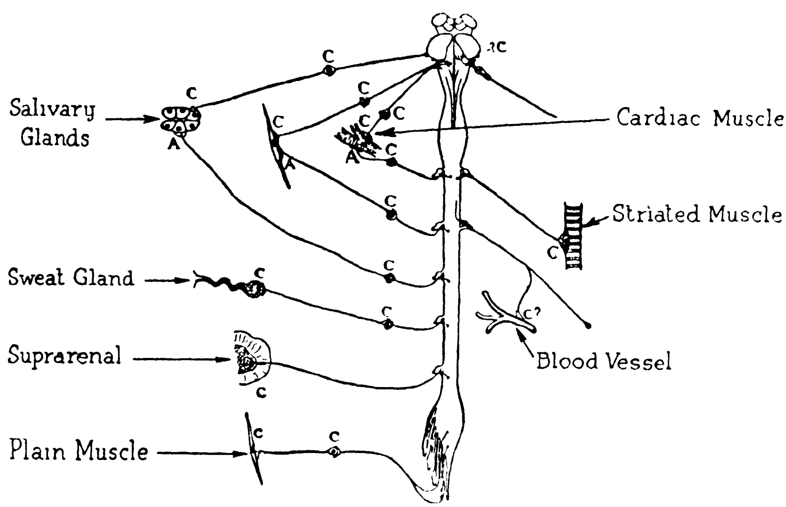

FIG. 232. Diagram of Peripheral Nervous System. At points marked C there is evidence of a cholinergic transmission, at those marked A, of an adrenergic transmission. (DALE. *B.M.J.*, 1934, (i.), 835.)

* CANNON and ROSENBLUETH. *Amer. J. Physiol.*, 1933, **104**, 557; 1935, **113**, **251**.
† FELDBERG and KRAYER. *Arch f. exp. Path.*, 1933, **172**, 170.
‡ FELDBERG and GADDUM. *J. Physiol.*, 1934, **81**, 305.
§ FELDBERG, MINZ and TSUDZIMURA. *J. Physiol.*, 1934, **81**, 286.

lionic autonomic fibres at their synapses in the ganglia or in the suprarenal, should be classed together under the name *cholinergic*, while those that liberate an adrenaline-like substance at their terminals should be called *adrenergic* (Fig. 232). The nerves to the sweat glands are cholinergic in some animals such as the cat, but adrenergic in others, *e.g.* the horse, though in all cases anatomically sympathetic and post-ganglionic.

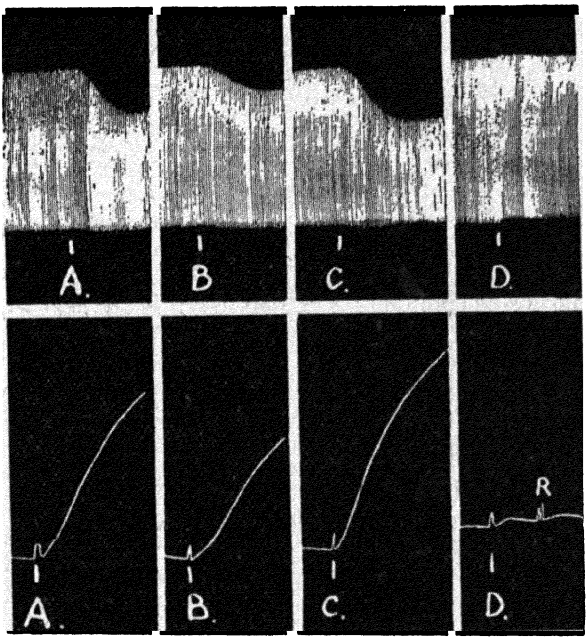

FIG. 233. Demonstration of Presence of Acetyl Choline in perfusate from the superior Cervical Ganglion of the Cat during Stimulation of the Cervical Sympathetic Nerve. Above, effect of perfusate from ganglion on perfused frog's heart; below, effect on eserinized leech. A. Fluid collected during stimulation; B. and C. Comparison by addition of acetyl choline (15γ and 30γ per l = 1 : 15 × 10⁹ and 1 : 30 × 10⁹ respectively). D = Control fluid without stimulation. (FELDBERG and GADDUM. *J. Physiol.*, 1934, **81**, 305.)

The actions of acetyl choline, like the effects of nerve stimulation, quickly pass away, and this is because the substance is rapidly destroyed by an enzyme, present in the tissues, and called choline-esterase * : in presence of eserine, however, the choline esterase is inhibited, so that the effect of acetyl choline is then more intense and prolonged. A usual test object for the detection of acetyl choline in the blood is the dorsal muscle of the leech, previously treated with eserine † ; this gives powerful contractions in the presence of minute traces of acetyl choline ($1 : 10^9$).

Acetyl choline rapidly disappears in alkaline solution. Those of its actions which simulate the action of the parasympathetic nerves are abolished, as the effects of stimulation of those nerves are also abolished, by the action of atropine. The effects known as the nicotine-like effects, however, *e.g.* the contracture of certain skeletal muscles, and the excitation of the cells of autonomic ganglia, are not paralysed by atropine.

* GLICK. *J. Gen. Physiol.*, 1938, **21**, 289 (estimation method).
† FÜHNER. *Arch. exp. Path. Pharm.*, 1918, **82**, 51, 205.

For purposes of physiological identification it is usual to consider the presence of acetyl choline as proved if the given solution causes contraction of the eserinized leech muscle, and has this property destroyed by previous treatment with alkali. Sometimes other tests are used instead or in addition; these are the contraction of the rectus abdominis muscle of the frog (by $1:10^8$), inhibition of the frog's heart (by 1 in 10^8), and fall of arterial pressure in the cat. The last two effects are abolished by atropine. By comparing these effects with those of solutions of acetyl choline of known strength, a rough quantitative estimation of the substance can be carried out. The use of these methods is illustrated in Fig. 233, which shows that the fluid perfusing a stimulated sympathetic ganglion contains acetyl choline.

The question arises whether the passage of nervous impulses across synapses in the central nervous system is also effected by the mediation of the same or some similar substances. The investigations of the Sherrington school on the subject of reflex action has led to the belief that the phenomena of recruitment, after-discharge and inhibition may be explained on the supposition that some central excitatory state, or some chemical substance causing such state persists at the synapse for an appreciable interval of time. On the other hand, if such were the case, it would be expected that the arrival of an impulse along a pre-ganglionic fibre might give rise to a prolonged activity in the post-ganglionic neurone, and that only after appreciable delay to allow for diffusion, &c. Yet it has been shown by Eccles that for each pre-ganglionic impulse there is one post-ganglionic impulse, and the delay is very brief at the synapse. Hence, if we are to accept the chemical transmission theory, it is evident that the rate of diffusion and subsequent destruction of the exciting substance must be very great. In view of the short distances to be traversed and of the extreme rapidity with which such substances as acetyl choline are destroyed by tissues, these objections need not be held to be insuperable.

Support for the chemical theory of transmission was provided by the demonstration that acetyl choline is liberated in a ganglion when the pre-ganglionic fibres to it are stimulated. Doubt has been thrown on these results, however, by Lorente de Nó,* who states that the phenomenon only occurs if damage has been done to the ganglion. There are various other unexplained phenomena in connection with the theory of chemical transmissions which show that some factors are at present unknown. Thus, atropine abolishes all the effects of acetyl choline, and nearly all the effects of parasympathetic excitation. But not all of them—for instance, atropine does not abolish the vaso-dilatation in the submaxillary gland on stimulation of the chorda tympani, though it does abolish the secretion, yet it abolishes both secretion and vaso-dilatation on administration of acetyl choline.

Ionic changes are certainly also concerned in the phenomenon. Thus the presence of K ions in the blood causes a release of acetyl choline from ganglion cells. but in absence of Ca ions K ions fail to do so; yet in absence of Ca ions the cells spontaneously discharge.†

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* Lorente de Nó. *Amer. J. Physiol.*, 1938, **121**, 331.

† Harvey and McIntosh. *J. Physiol.*. 1940, **97**, 408.

LOEWI (1935). Problems Connected with the Principle of Humoral Transmission of Nerve Impulses. *Proc. R. S.*, **118B**, 299.
NEWTON (1939). Recent Advances in Physiology, 6th Ed., p. 352. (London: Churchill.)
ROSENBLUETH (1937). The Transmission of Sympathetic Nerve Impulses. *Physiol. Rev.*, **17**, 514.

(3) AFFERENT FIBRES OF THE AUTONOMIC SYSTEM

There are abundant afferent fibres, many of them non-myelinated, in all the autonomic nerves. Thus, stimulation of the cut central end of the splanchnic nerve produces reflex effects such as a dilatation of the pupil, rise of blood pressure, &c. The autonomic afferent fibres come from all parts of the body, and commence in receptors of various types in the viscera and somatic structures.

Spinal autonomic afferents pass from the periphery in the splanchnic nerves, phrenics, &c., and some also with the various spinal nerves. They pass without interruption through the sympathetic ganglia, and then by the white rami communicantes * reach the dorsal roots, where their cell bodies are situated, as for the somatic afferents ; like these they enter the dorsal roots of the spinal cord (Fig. 234). The spinal roots concerned are all those that have white rami, viz. the thoracic and upper 3 lumbar and the 2nd to 4th sacral roots.

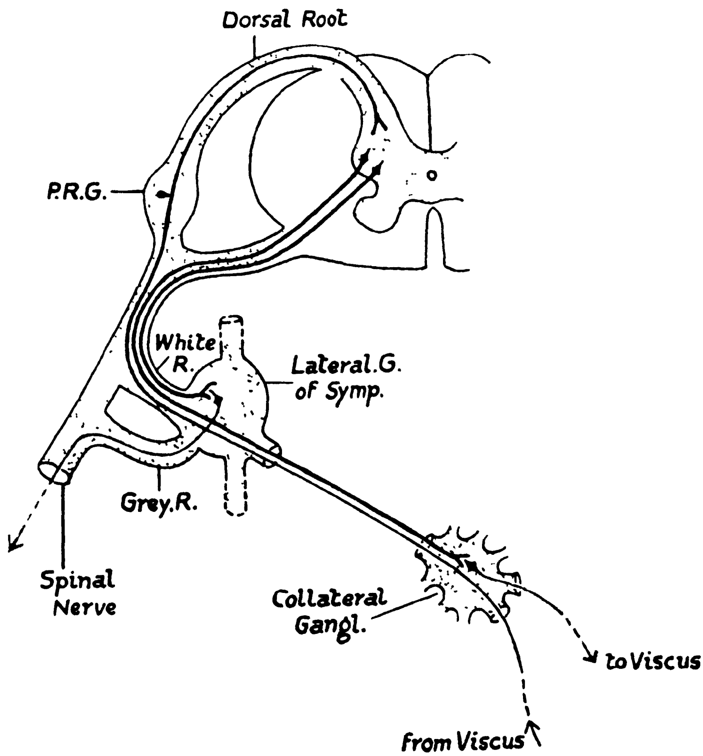

FIG. 234. Diagram showing the Afferent and Efferent Connections of the Sympathetic System. Afferent fibres blue, preganglionic efferents black, post-ganglionic efferents red.

* RANSON and BILLINGSLEY. *J. Comp. Neurol.*, 1918, **29**, 405.

Having entered the spinal cord the afferent autonomic fibres probably branch, much as the somatic afferent fibres do, and some terminate in relation to cells of the dorsal horn of grey matter. From here the further path may diffuse widely, by a series of relays, but sooner or later arcs are completed by some of the collaterals, which form synapses with cells in the intermediolateral cell columns in the cord. From these cells the efferent sympathetic fibres emerge.

How widely the efferent effects of afferent autonomic stimuli may spread is shown by the fact that on stimulation of the intestine by stretching, dilatation of the pupil occurs.*

Referred Pain. The afferent fibres from the viscera are of importance in connection with visceral pain. To most types of stimulation such as cutting, burning, &c., the viscera are quite insensitive, and normally we get no sensations at all from them, but pulling on the mesentery, or distension of hollow organs gives rise to pain which is usually 'referred' to a definite area of the skin. These areas, which become hypersensitive, are constant for each organ. Thus, distension of the cæcum or appendix results in pain referred to the right iliac fossa, over-distension of the heart and aorta to the pain of angina pectoris which radiates down the left chest, arm and shoulder, and so on. The explanation is that the pain is referred to that area of skin supplied with ordinary somatic sensory fibres derived from the same spinal roots as provide the afferent fibres for the viscus concerned. Thus the afferents from the heart enter by Th. 1–4. Also the afferents from the skin of the chest and inner side of arm enter by those roots. Since impulses from the heart do not normally enter consciousness at all, any that exceptionally,

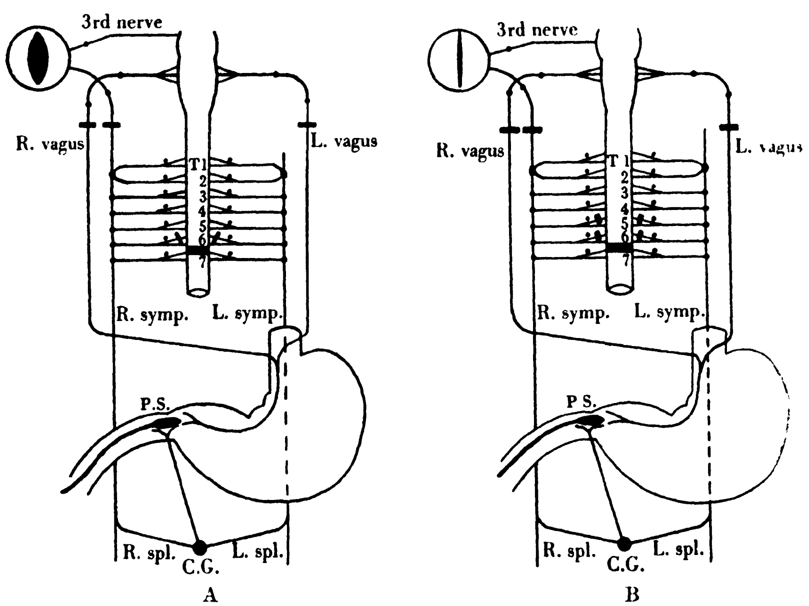

FIG. 235. Determination of Upper Limit of Entry of Afferents from Pyloric Sphincter. A. Cord cut between 6th and 7th Th. segments. R. and L. vagi and R. and L. cervical sympathetic nerves cut in the neck; 6th Th. dorsal roots cut. Dilatation of pupil on distension of pyloric sphincter. B. On now cutting 5th Th. dorsal roots the response vanishes. C G = cœliac ganglion (McSWINEY and SUFFOLK. *J. Physiol.*, 1938, **93**, 104.)

* McDOWALL. *Q. J. Exp. Physiol.*, 1925, **15**, 177.

do so are accepted as coming from the skin area supplied by the somatic afferents.

A method by which to find the levels of entry of autonomic afferents into the cord has been worked out by Irving and McSwiney *. When any part of the alimentary canal is distended by a balloon, a reflex rise of arterial pressure and dilatation of the pupil by inhibition of tone of the IIIrd nerve occurs. By making sections of different parts of the central nervous system until this reaction disappears the path of entry and transit can be found. Fig. 235 illustrates the procedure for distension of the pyloric sphincter, and shows that the afferents enter below the fifth thoracic root. By analogous procedures it was found that the lower limit for pyloric sphincter afferents was the thirteenth thoracic root.

Cranial autonomic afferents are contained in the IIIrd, VIIth, IXth, Xth and XIth cranial nerves and perhaps in others, but it is not always possible to differentiate clearly between autonomic and somatic afferents. They have cell bodies in ganglia similar to the dorsal root ganglia, on the course of the nerves, and after entering the brain stem terminate in relation to cells of one of the afferent nuclei. Thus the afferents in the *vagus* are the axons of cells in the ganglion jugulare and ganglion nodosum. Their centrally running portions enter the medulla and branch, some of the branches ending in the dorsal vagal nucleus, in the floor of the IVth ventricle, in relation to cells the axons of which pass out in the vagus again as efferent autonomic fibres. This is a simple type of autonomic reflex arc, and many similar ones will be found in chapters dealing with the functions of the various viscera.

Another important example of an autonomic afferent is the nerve from the *carotid sinus*, a branch of the IXth cranial nerve. It conveys impulses resulting from stretching of the carotid artery and from changes in the composition of the blood therein, and important circulatory and respiratory reflexes are thereby brought about.

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THE INTEGRATION OF AUTONOMIC FUNCTIONS

It is evident that, in the main, the actions of the sympathetic and parasympathetic systems are opposed to one another, and many viscera have both supplies. Generally, the sympathetic system brings about changes that enable the organism to react to emergencies in such a way as to maintain a constant environment for its cells, or to help to protect itself by fight or flight, by bringing all its reserves into action, even at some cost. The parasympathetic system is similarly credited with more conservative functions, slowing the heart, reducing light to the eye, aiding digestion and generally serving a recuperative function.

It is perhaps more useful, however, to regard these two systems as both playing their parts in maintaining uniformity of conditions for the tissues of

* IRVING and MCSWINEY. *J. Physiol.*, 1935, **85,** 4P.

he body, by reason of their activities being balanced one against the other 1 such a way as to enable the viscera to work within a very wide range ccording to the varying requirements. The exact adjustment of body emperature or local blood supply, for example, requires a delicately balanced o-operation of both sympathetic and parasympathetic activities, and hysiological enquiry is in fact very largely occupied in unravelling such o-ordinated activities, examples of which may be found in all branches of he subject.

It would appear that there are levels of integrative function in the utonomic system, as in the somatic system, and here also these become of icreasing complexity as we go higher in the central nervous system.

There are autonomic vasomotor, pilomotor and sweat centres in the ioracic spinal cord, and centres controlling pelvic organs in the lumbar nd sacral cord, through all of which simple reflexes may be obtained ivolving only the spinal cord, *e.g.* a rise of blood pressure on stimulating ie central end of the splanchnic nerve in the spinal cat.

In the various afferent and efferent nuclei of the medulla and mid-brain e have not merely centres for the carrying out of special reflexes with which iose efferent nuclei are concerned, *e.g.* the salivary nuclei in the lower pons nd upper medulla, a vasomotor centre and a cardiac centre in the lower iedulla, but also there are co-ordinating centres which exert an effect on ie centres that lie lower down. Thus the medullary vasomotor centre tercises control on the spinal vasomotor centres, a centre in the mid-brain concerned in co-ordinating the spinal reflexes of micturition, &c. More omplex reflexes, *e.g.* swallowing, or salivation, as well as extensive circulatory id respiratory reflexes can be seen in the decerebrate cat, but this animal ill lacks extensively co-ordinated reflexes, *e.g.* it cannot regulate its body mperature, or its blood sugar level.

When the hypothalamus is left behind, however, as in the thalamic animal, ie body temperature and other autonomically regulated characters remain ormal, and it is now generally agreed that it is in the hypothalamus that ie highest refinements of autonomic adjustment are effected. There is in ldition some degree of voluntary cortical control of autonomic function, g. in evacuation of the bladder, modification of breathing, &c., but this superadded and does little to modify the integrated result of the hypo-ialamic functions.

II. THE HYPOTHALAMUS

The hypothalamus, which lies at the base of the diencephalon, below the ialamus, forms part of the wall and floor of the third ventricle. As seen om the base of the brain, it occupies the interpeduncular space and imprises the tuber cinereum and hypophysis anteriorly and the corpora ammillaria posteriorly; between the latter, and forming the floor of the ird ventricle is the 'central grey matter.' Dorsally, it is separated from ie thalamus by a fissure. The hypothalamus has an abundant blood supply.

Among other functions the hypothalamus has important connections ith the olfactory tracts.

The hypophysis is an endocrine organ, which will be further discussed in later chapter; it has important functional relations to the other hypo-alamic structures.

Hypothalamic nuclei. Scattered throughout the hypothalamus are imerous nuclei and certain tracts. The nuclei and their connections are

complex, and have not yet been fully worked out, but Le Gros Clark * has outlined a classification, as follows :—

(1) *Anterior Group*, above the optic chiasma,
 (*a*) The paraventricular nucleus, near the IIIrd ventricle.
 (*b*) The supra-optic nucleus, just above the optic chiasma.
(2) *Middle Group*, related to the infundibulum and tuber cinereum.
 (*a*) Ventro-medial nucleus.
 (*b*) Dorso-medial nucleus.
 (*c*) Lateral nuclei.
 (*d*) N. tuberalis.
(3) *Posterior Group*, related to the mammillary bodies,
 (*a*) Posterior nucleus, above mammillary bodies.
 (*b*) Corpus mammillare nuclei.

Some of these nuclei are shown diagrammatically in Fig. 236.

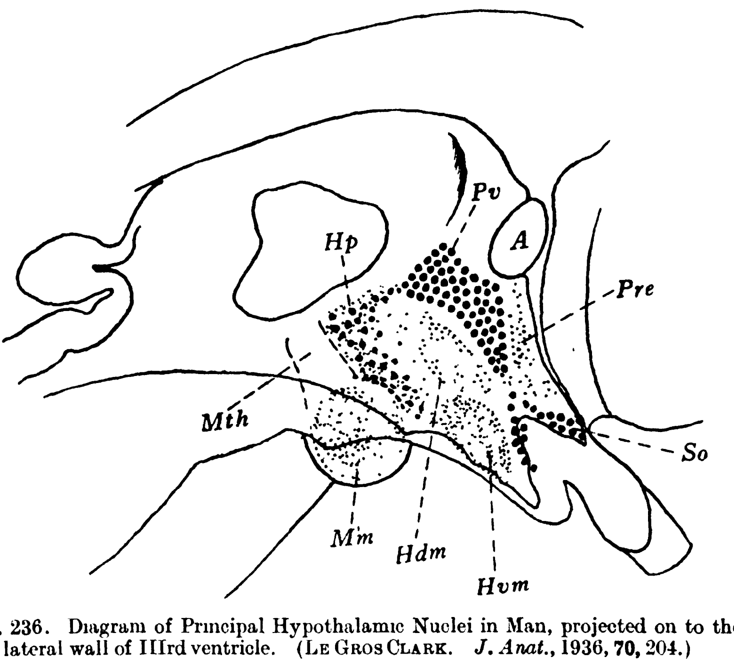

FIG. 236. Diagram of Principal Hypothalamic Nuclei in Man, projected on to the lateral wall of IIIrd ventricle. (LE GROS CLARK. *J. Anat.*, 1936, **70**, 204.)

Anterior Group.	Pv. Paraventricular N. So. Supra-optic N.	A. Ant. Commissure.
Middle Group.	Hvm. Ventromedian N. Hdm. Dorsomedian N.	
Posterior Group.	Hp. Posterior N. Mm Mammillary nuclei	Mth. Mammillothalamic tract.

CONNECTIONS. There are many short connections, not yet fully mapped out, between the various hypothalamic nuclei and with adjacent structures. There are also longer afferent and efferent connections.

Afferent fibres reach the hypothalamus from

(1) The olfactory tract. From the hippocampus *viâ* the column of the fornix to the mammillary bodies.

(2) Thalamus to posterior nucleus.

* LE GROS CLARK. *J. Anat.*, 1936, **70**, 203.

(3) Various parts of the body, *e.g.* from vagus, various ascending tracts in mid-brain, etc.

(4) Cortex, *viâ* unknown paths (not definitely proved).

Efferent connections from the hypothalamus pass to

(1) The thalamus, from the mammillary bodies (the mammillothalamic tract is the most conspicuous tract in this region).

(2) The cortex, *viâ* the thalamus.

(3) autonomic nuclei of the brain stem and spinal cord (intermediolateral cell column). This is the most important of the descending efferent tracts : it probably starts in the lateral nuclei of the hypothalamus and leads to the reticular formation of the brain stem *, from which a series of relays passes to the efferent neurones from which arise the pre-ganglionic autonomic fibres. The connections are largely uncrossed, or if crossed, become so quite near their endings in the cord †.

(4) The hypophysis, by way of the supra-optico-hypophyseal and tubero-hypophyseal tracts which run in the stalk to reach the posterior lobe of the pituitary body (Fig. 237).

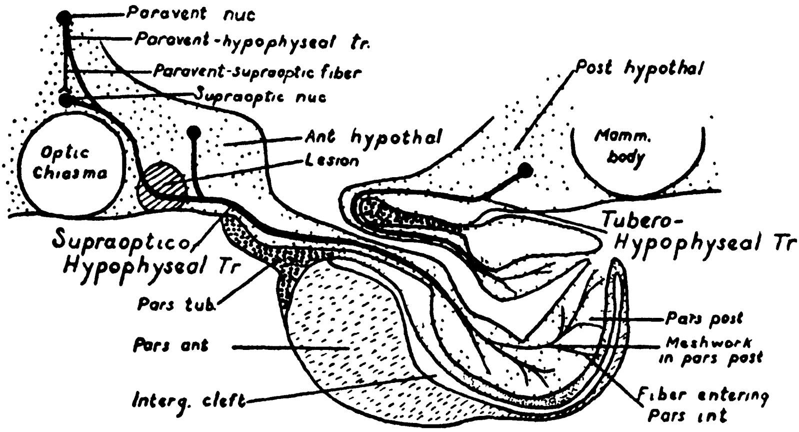

FIG. 237. Diagram of Medial Saggital Section of Hypothalamic Region of Cat showing Nervous Connections of Posterior Lobe of Pituitary Body. (RANSON, Harvey Lecture, 1936–37.)

FUNCTIONS OF THE HYPOTHALAMUS

Stimulation. It was found by Karplus and Kreidl in 1908 that on electrical stimulation of the hypothalamus there was widespread excitation of the sympathetic nervous system ; Beattie, Brow and Long ‡ in 1930 produced evidence that there were tracts connecting the hypothalamus with the spinal sympathetic centres ; and in 1935 Beattie and Sheehan § showed that both sympathetic and parasympathetic effects (*i.e.* reduction or increase of gastric motility) could be produced by suitable excitation, in the former case of the posterior nuclei and in the latter of the tuber region. Detailed studies of the results of strictly localized excitation of known points in the hypothalamus have been carried out by Ranson and Magoun and their colleagues,‖ and much precise information obtained.

* BEATTIE, BROW and LONG. *Proc. R.S.*, 1930, **106B**, 253.

† HARRISON, WANG and BERRY. *Amer. J. Physiol.*, 1939, **125**, 449.

‡ BEATTIE, BROW and LONG. *Proc. R.S.*, 1930, **106B**, 253.

§ BEATTIE and SHEEHAN *J. Physiol*, 1934, **81**, 218.

‖ RANSON. *Ergebnisse d. Physiol.*, 1939, **41**, 56.

In sum, the results have shown that general sympathetic responses, such as cardiac acceleration, rise of blood pressure, pupil dilatation, pilo-erection, hyperglycæmia, inhibition of bladder and intestinal tonus, resulted from stimulation of the posterior and lateral group of hypothalamic nuclei. Increase of respiration rate also occurred. Stimulation of the middle group of nuclei, in the tuber region, caused parasympathetic responses, *e.g.* bladder contraction, cardiac inhibition, and increased motility of the alimentary canal; it also caused sweating. Anterior group stimuli also caused some parasympathetic effects of doubtful significance and possibly caused changes in the water balance of the body. These last might be related to effects on the pituitary.

When the hypothalamus was stimulated in a conscious cat by electrodes fixed into the skull, all the symptoms of intense emotional excitement resulted, accompanied by powerful sympathetic effects, and it will be recalled that outbursts of 'sham rage' with intense sympathetic excitation are also seen in decorticate cats and are explained as release phenomena due to removal of cortical control.

Injection into the lateral ventricles of the brain of pituitary extracts, pilocarpine or acetyl choline appears to stimulate certain hypothalamic nuclei, causing general sweating, vasodilatation, salivation, vomiting, &c.*

Lesions. Many remarkable effects have been found to be associated with lesions of the hypothalamus. They fall roughly into two groups, viz. those closely related to the functions of the pituitary body and those with purely nervous effects. As regards the former group, it is still not clear where the functions of the pituitary body, which are mainly endocrine, cease and those of the hypothalamus begin, and many of the effects of lesions of the one are also found when damage is done to the other.

In one case the relation is particularly clear, viz. in the regulation of water balance. Removal of the posterior lobe of the pituitary causes the condition of *diabetes insipidus*, characterized by the passage of enormous volumes of urine. But lesions in the neighbourhood of the supra-optic nuclei, or in the pituitary stalk, also cause polyuria, and this appears to be due to interference with the tract connecting this nucleus with the posterior pituitary.† It may be inferred from this that the supra-optic nuclei exert control over the formation, by the posterior pituitary, of the hormone which controls excretion of water by the kidney.

Other functions are also related to the anterior pituitary body; thus injury of the tuber causes sexual dystrophy with adiposity, injury to the anterior region affects carbohydrate metabolism causing a lowering of blood sugar, &c. Whether such injuries produce these effects by interfering with an innervation which controls the formation of the responsible hormones in the pituitary body is at present uncertain.

The more purely nervous effects of lesions of the hypothalamus are shown by an upset of various intricately co-ordinated functions. One of these is temperature regulation, which will be further discussed in a later chapter. It need only be stated here that hypothalamic lesions, according to their extent and position may cause an animal to lose the ability to withstand heat, cold or both.

Another complex effect of lesions is the production of a state of somnolence. As pointed out in Chapter XXI, the hypothalamus, and especially its posterior

* CUSHING. "The Pituitary Body, Hypothalamus, etc." 1932. (Thomas, Springfield, Ill.)

† FISHER, INGRAM and RANSON. "Diabetes Insipidus and the Neuro-hormonal Control of Water Balance." 1938. (Edwards Bros. Ann Arbor.)

nd lateral portions, directs the activities of those organs and tissues which re aroused in times of emergency, and the withdrawal of this drive results n emotional apathy or somnolence. It is perhaps more correct to take this iew than to postulate a 'sleep' centre in this part of the brain.

Lastly, it has been found in man that psychic disturbances are common in atients with lesions of the hypothalamus. These disturbances may take he form of acute mania, or, more rarely, of somnolence and lassitude.

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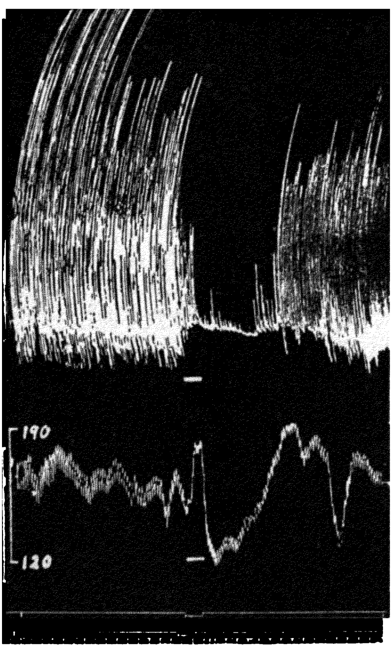
'ULTON and RANSON (1940). The Hypothalamus and Central Levels of Autonomic Function. (Baltimore: Williams & Wilkins Co.)

CORTICAL CONTROL OF AUTONOMIC FUNCTIONS

There is abundant evidence that autonomic functions are subject to ome degree of control from the cerebral cortex. This is not surprising, ince autonomic activity so often accompanies that which is controlled from he cerebrum. In general, the autonomic functions are controlled from areas f the cortex which are concerned with the somatic activities of adjacent tructures. Hence, when the motor area for eye movements is stimulated, upil changes, and lachrymation results, or if that concerned with tongue novements is excited, salivation is seen. Vascular responses, *e.g.* vaso-constriction or vaso-dilatation are obtained from certain points on areas 4 and 6, stomach and intestine movements, and sweating, from area 6, &c. These cortical areas appear in part to exert their effects *viâ* the hypothalamus, but partly by other extra-pyramidal tracts.



'IG. 238. Chloralosed Cat Knee-jerks elicited every ten seconds by tapping patellar tendon (upper tracing) Blood pressure in carotid artery (lower tracing). At the signal, stimulation of central end of left vagus. (SCHWEITZER and WRIGHT. *J. Physiol.*, 1937, 88, 459.)

THE IRRADIATION OF AUTONOMIC REFLEXES

We have seen that somatic reflexes tend to be directed with some precision, and often to structures closely related to the site of a stimulus. Nevertheless, there is also a tendency for the effects to spread or irradiate to other structures, usually in a definite order.

In the autonomic system, irradiation is a much more pronounced phenomenon. Indeed, as regards the sympathetic system, the effect of an afferent impulse is to set the whole sympathetic system into activity, and its structure is well suited for such widespread responses. Hence, for example, if the central end of the splanchnic nerve is stimulated, the effects reach even to the pupil, which

dilates. In the parasympathetic system there is less irradiation than in the sympathetic, but it is still well marked. Thus if the central end of the depressor nerve is stimulated, not only is the heart slowed, but also the peristaltic movements of the intestines are augmented.

Usually the sympathetic and parasympathetic systems act reciprocally, an increase of the one being accompanied by a reduction in the activity of the other. So, for instance, if the nerve to the carotid sinus (Chapter XXXIV) is stimulated, the heart is slowed, partly because the vagus tone is increased and partly because the sympathetic tone is diminished.

Not only do autonomic reflexes irradiate over the whole autonomic system, but the spread also involves the somatic reflexes. Thus, stimulation of the central end of the vagus nerve causes reduction or abolition of the knee-jerk, and of other somatic reflexes (Fig. 238).

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BOOK IV

THE SPECIAL SENSES

(By H. HARTRIDGE, M.D., Sc.D., F.R.S.)

CHAPTER XXIV

THE NERVOUS MECHANISM OF SENSATION

GENERAL PHYSIOLOGY OF SENSATION

THE ability to originate sensations is common to almost all parts of the body. Thus the muscles, joints and viscera send nerve impulses to the brain, which records their activities. We can classify the sense organs into two main groups, those which belong to the common sensibility of the body, and those which form the special senses. The characteristics of the former are (1) that rarely do their impulses, other than those of pain, ever pass the threshold of consciousness ; (2) that they play a very large part in the initiation of the numerous reflex actions ; (3) that when their impulses do reach consciousness they lack definition. The characteristics of the special senses on the other hand are :—(1) that most of their nerve impulses reach consciousness ; (2) that the connection between the stimulus and the resulting response on the part of the individual (as expressed by movements of limbs, &c.) is usually not of the simple nature of a spinal reflex ; (3) that the information supplied to consciousness is very definite both as to quality and intensity.

The indefiniteness of the connection between stimulus and response causes us to depend almost entirely on introspection in our study of the sense organs. But introspection as a method of research has grave disadvantages, for we cannot measure our sensations in terms of physical units. We cannot say how red a rose is or how nice it smells. Neither can we compare the intensity of a beam of light, when we feel its warmth on our skin, with the strength of the stimulus which we receive when it enters our eye. Still less can we judge accurately of the sensations evoked in other individuals or animals when we apply stimuli to them. Because of this difficulty of measuring sensations, two methods of investigation have been developed which to a great extent avoid it, namely the *method of threshold values* and the *method of comparisons*.

In the method of threshold values, gradually increasing stimuli are applied to the sense organ under investigation until a sensation is just perceived. Thus, increasing weights are applied to the skin until their pressure is felt, or a light of increasing intensity is presented to the eye until its presence is seen. Such threshold values give definite information concerning the sense organ to which they refer, and allow us to compare the same effects in one and the same sense organ under different conditions and also in different individuals.

In the method of comparisons two separate stimuli are applied, and one or both are varied until the sensations caused by them are equal. Thus a ray of yellow light may be compared with a ray mixed from red and green light, and the intensities of the two adjusted until the sensations caused by both are similar. Both these methods give concordant results if care be taken to make the conditions standard, and both are for this reason largely used for studying the sense organs.

A careful study of these organs is necessary and important because it is

only through their agency that we derive information about ourselves, one another, and the world in which we live. The limitations of our sense organs, therefore, restrict our knowledge of the many transformations of energy that are going on around us, except in so far as we are able to devise means of extending them artificially. Thus our knowledge of the existence of ultra-violet light began only with the discovery of photography. Wireless waves circulated through the ether around unknown to us before the invention of the coherer.

CLASSIFICATION OF SENSE ORGANS. Sense organs differ in their anatomical position and structure, and also according to the nature of the stimulus to which they react and the kind of sensation which they cause. Any of these differences might be used as a basis of classification, but the latter is found to be the best.

Structure.	Sensations.	
Skin	Touch, Pain, Heat, Cold	Feeling
Eye	Light, Colour, Shape, Distance	Sight
Ear	Pitch, Intensity, Position	Hearing
Tongue	Acid, Sweet, Bitter, Salt, Alkaline	Taste
Nose	Various	Smell

Stimuli adequate for one sense organ are found to be inadequate for another. Thus sound applied to the ear is adequate, but to the eye is not.

The sense organs appear to be elaborated from a very simple type, and the higher they are developmentally, the more is one kind of stimulus adequate and the less are all others.

Thus the end organs in the cornea react alike by a sensation of pain to touch, electricity, heat, inflammation, &c., while the ear reacts to sound waves only. This specific property of the end organs is also attended by an increase in the strength of response to the chosen stimulus. Thus the retina of the eye can be stimulated mechanically and electrically, but has its greatest sensitiveness to light. It is estimated that the eye is many times as sensitive to light as any instrument that has been so far constructed. This specialisation of the end organs makes the information which we obtain from them more detailed and complete, but at the same time sets a limitation to the range of stimulus to which each can respond. Thus a pain end organ can react alike to heat rays, visible rays and ultra-violet rays, while the retina of the eye responds to visible rays only.

THE LAW OF SPECIFIC IRRITABILITY. A very little consideration suffices to show that there is no resemblance between a sensation and the stimulus, and that one and the same physical event applied to different sense organs will evoke absolutely distinct sensations. If we take a tuning-fork which is vibrating 100 times per second and apply it to the surface of the skin, we get simply a sensation of vibration, *i.e.* a series of tactile

impressions repeated at rapid intervals. If the same tuning-fork be applied to the head, its vibrations are imparted to the bones of the skull and thence to the auditory nerve endings and arouse in our consciousness the sensation of a certain musical note. The same thing happens if the vibrations of the tuning-fork are conducted by the ear to the auditory nerve endings in the ordinary way through the external and middle ear. On the other hand, it can be shown that different kinds of stimuli applied to one sense organ always evoke the same kind of sensation, if any. Thus a sensation of light may be aroused not only by the incidence of radiant energy of a certain wave length on the retina, but also by electrical or mechanical stimulation of the retina. If the eye be turned inwards and the finger be pressed on the eye through the outer canthus of the lids, a sensation of light is aroused and we see a coloured circle which we refer to some spot lying to the nasal side of the eye stimulated. The character of the sensation therefore bears no resemblance to the physical events by which the sensation is evoked, but depends entirely on the nature of the sense organ which is stimulated. A sensation of light may be produced by any stimulation of the retina, or of the optic nerve, or of the terminations of the optic nerve in the brain. In the same way stimulation of an auditory nerve or its intracranial endings gives rise to sensations of sound.

Where the question has been investigated, it has not been found possible to evoke different qualities of sensation by different modes of stimulation of nerve fibres. It has therefore been concluded that the quality of any sensation depends simply and solely on the termination of these nerves in the central nervous system, and that where sensations of different quality are produced there must also be difference of nerve paths. This idea was formulated by Müller, and is often alluded to as Müller's 'law of specific irritability.' The law states that every sensory nerve reacts to one form of stimulus and gives rise to one form of sensation only ; though, if under abnormal conditions it be excited by other forms of stimuli, the sensation evoked will still be the usual one.

LOCALISATION. Although the different forms of sensation must be regarded as dependent on the integrity of the brain and of its connections with the peripheral sense organs, sensations are not referred to the brain, but are localised as proceeding from some part of the body or from some region outside the body. Thus the sensation of taste is always localised in the mouth ; sensation of touch at the skin or surface of the body ; while the sensations of hearing and of sight are 'projected,' *i.e.* are interpreted as coming from the environment outside ourselves. Even the organic sensations of posture or fatigue are referred to the peripheral reacting parts of the body and not to the central nervous system. A sensation, therefore, cannot be interpreted as a reproduction of external events, but as a *symbol* of these events evoked by stimulation of the sense organs of the body.

RELATIONSHIP BETWEEN STIMULUS AND SENSATION. Although the sensation is not a reproduction of the stimulus, it is a symbol of the stimulus, and can be used to inform us of events occurring in the world around. Like stimuli, falling on the same end organ, always evoke like sensations, other conditions being equal. An orderly sequence of sensations may therefore be interpreted as indicating a corresponding orderly sequence of physical occurrences in the world around us. Since our sensations are merely symbols of the physical conditions which give rise to them, it is important to inquire how far they correspond *quantitatively*.

THRESHOLDS. Whatever form of stimulus be applied and whatever sense organs be affected, a certain minimum intensity of stimulus is necessary for it

to be effective, *i.e.* to produce a minimum sensation. This strength, which varies with different sense organs, is spoken of as the 'liminal intensity' or 'threshold value' of stimulus or sensation respectively. In each sense organ we can measure the amount of energy which must be applied to it in order to evoke a minimum sensation. This figure varies considerably with the physiological condition of the subject. Thus it is found to vary in most cases with the following factors :

(1) The rate of application of the stimulus.

(2) The method of average error in matching.

(3) Events occurring in the surrounding areas, *i.e.* simultaneous contrast.

(4) Events which have previously occurred in the area receiving stimulation, *i.e.* successive contrast.

(5) The time during which the stimulus acts, *i.e.* adaptation.

WEBER'S LAW. It is an interesting question how far the strength of sensation may be regarded as an index to the strength of stimulus. Although it is easy to measure in absolute terms the intensity of a stimulus, and although we can say that such and such a light is stronger than another light, it is impossible to say that the sensation resulting from the stronger is two, three or more times that of the weaker. In measuring the effect on sensa-

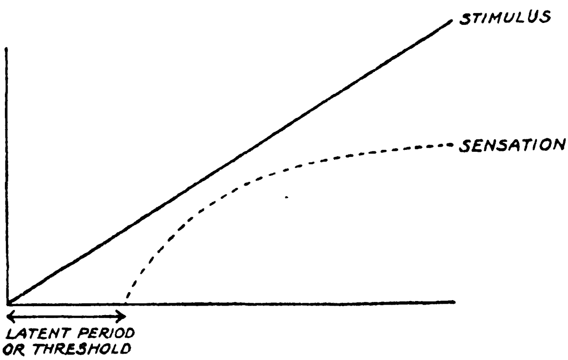

FIG. 239. Diagram to show Relationship between Stimulus and Sensation.

tion of increasing the stimulus, we are therefore reduced to using the *smallest appreciable increase of sensation* as our unit of sensation. Weber's law states that the increase of stimulus, which is necessary to produce an appreciable increase in sensation, always bears the same ratio to the whole stimulus. Thus, if we found that we could just distinguish the difference between a weight of 20 g. and a weight of 21 g., it would not be sufficient to add 5 g. to a weight of 200 g. in order to produce a distinct difference in sensation. In the latter case we should not be able to appreciate any difference until we had added 10 g., *i.e.* one-twentieth of the whole stimulus to the weight.

Several methods have been proposed for testing the limits of the applicability of this law. Of these the most important are :

(1) The method of minimal difference.

(2) The method of average error in matching.

In the first method we find by trial how much a given stimulus must be increased in order to evoke an appreciable increase of sensation, and this determination is made for a number of stimuli of different intensities. In the second method it is sought to find a strength of stimulus which is just

equal to another stimulus of given intensity. It will be found that errors will be made on both sides, and the average error is taken as representing the minimum difference which is just sufficient to cause a distinct difference of sensation.

In all sense organs Weber's law is applicable only between limits which vary with each sense organ, and it does not hold either for very weak or for very strong stimuli. Within these limits the ratio, which an increase of stimulus must bear to the whole stimulus in order to produce an increase of sensation, may be given approximately as follows for the different sense organs :—

Sense Organ.	*Quality determined.*	*Ratio.*
The ear	Difference of intensity	$\frac{1}{9} - \frac{1}{20}$
The eye	Difference of intensity	$\frac{1}{167}$
The skin	Difference of weight	$\frac{1}{20} - \frac{1}{40}$

FECHNER'S LAW gives the result of an attempt to state Weber's law in mathematical terms. It states that the sensation varies as the natural logarithm of the stimulus. This relationship is shown diagrammatically in Fig. 239.

CHAPTER XXV

VISION

1. PHYSICAL PROPERTIES OF LIGHT

The Spectrum. It is possible by suitable apparatus to cause the constituent rays in a beam of light to arrange themselves according to their wavelength. When thus arranged they are said to form a spectrum. The apparatus is therefore called a spectroscope. The visible rays thus arranged are seen as a coloured band which has the following appearance. Visibility usually begins at about 8000–7800 A.U., the rays of longest wavelength being red. As the wavelength becomes shorter the colour gradually changes to orange, the transition being at about 6500 A.U. From orange the colour changes to yellow, at about 6000 A.U., from yellow to green at 5500 A.U., to blue-green at 5000 A.U., to blue at 4500 A.U., and to violet at 4000 A.U. The violet extends to 3800 A.U., where visibility ceases. The spectrum exhibits therefore a gradual change of colour with wavelength. Above the red is the invisible region occupied by the infra-red or heat rays, and below the violet the invisible ultra-violet or actinic rays, as explained above.

The colours of the spectrum have important properties which form the foundation of the science of colour mixture. If the spectrum produced from white light is caused to fold up again, it is found that white light is re-formed. But white light is produced if certain pairs of colours only are caused to combine in the correct proportion. Thus red (6562 A.U.) and blue-green (4921 A.U.) when mixed correctly form white light, so also do yellow (5636 A.U.) and violet (4330 A.U.). Such pairs are called *complementary colours*. But since there is in the spectrum a gradual transition from one colour to the next, so there are between red and yellow an infinite number of rays of different wavelength, each of which has its complementary colour, between blue-green and violet. If, therefore, from white light we remove one of a pair of complementary colours, the other member of the pair will be left unneutralised, and the light thus becomes tinted with its colour. Green rays do not possess a complementary in the spectrum; but it is found by experiment that, by combining red and violet to form purple, the required colour may be produced. If we include purple with the spectral colours, we can imagine these colours to form a closed ring. Each colour will then have its complementary opposite to it.

The Spectral Colours have another important property, for if red and yellow are caused to combine, they are found to produce orange, the intermediate colour. If red and green are mixed, then again the intermediate colour, yellow, may be produced. It is found that by varying the intensities of the two components, it is possible to produce orange or yellow-green, or in fact any other intermediate colour at will. Careful experiment shows that the intermediate colour thus formed is no mere approximation but an exact match. If red and green are thus able to combine to form the intermediate colour, while red and blue-green are complementaries producing white by their mixture, the question arises as to the effect produced by mixing red with a colour intermediate between green and blue-green. Experiment shows that a range of colours is produced containing an amount of white light, which varies with the intensities and wavelengths of the combining colours. Colours diluted with white light are spoken of as *unsaturated*. In order that the colours produced by a mixture of red and green rays shall be fully saturated, and thus be able to match the colours of the spectrum exactly, the green must not be shorter in wavelength than 5400 A.U. Similar phenomena are to be found at the other end of the spectrum; green and violet, when mixed in various proportions, form colours which match the intermediate spectral colours. With red, green and violet it is therefore possible to match the whole spectrum. But since red and violet, when mixed, form the intermediate purples, it is possible with the three coloured rays to imitate the whole range of colours. Now the purple formed from red and violet is, as we have seen, the complementary colour to green; by means of these three colours it is thus possible to produce white light. It should therefore be possible to match an unsaturated colour as easily as a saturated one. Experiment shows that such is the

case. The third property, beside colour and saturation, is *intensity*, which depends on the amplitude of the waves. The intensity of the mixture formed by red, green and violet, can therefore be readily adjusted by varying the intensity of each of the three component rays. We may summarise the above facts by stating that, by varying the intensities of the red, green and violet rays, it is possible to match every shade and colour. This statement has been put to the test by Maxwell, Abney and other observers, and has been found to hold good in all cases but one, spectral blue being slightly more saturated than the mixture of green and violet. In describing the complementary pairs of colours, we have mentioned that if the spectral colours are placed in a closed ring, complementary pairs are found to be opposite to one another. If now the three fundamental colours are placed at equal intervals round the ring, we may regard white as occupying the centre, because it is equidistant from the three fundamentals, and at the same time lies on the diameter between the various colours and their complementaries. If the other spectral colours are arranged in position relatively to the three fundamentals, they form a figure that in shape resembles a triangle more closely than it does a ring. This is due to the facts already mentioned (1) with regard to the exact matching of the spectral colours between red and green, by mixtures of

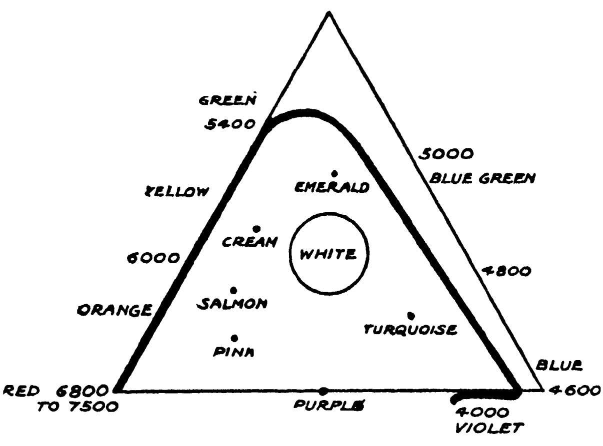

FIG. 240. Colour Triangle.

The black line shows the shape of the curve along which the different rays of the spectrum fall for white to occupy the central position.

those two fundamental colours; (2) with regard to the approximate matching of the region between green and violet, by the mixtures of those colours; and (3) with regard to the exact matching of mauves and purples, by mixtures of red and violet. The colour triangle which is shown in Fig. 240 therefore has a purely experimental basis, and has no association whatever with theories of vision.

PHOTO-CHEMICAL CHANGE, which is the third property of light mentioned above, is well illustrated by photography. The most important principle of light action is that light, to cause chemical change, must be absorbed (Draper's law). For example, an ordinary photographic plate which is found to be opaque to blue, violet and ultra-violet rays, and to be transparent to the rest of the visible spectrum, is therefore sensitive to the former rays but inactive to the latter. Further, by colouring the plate by a dye which absorbs red, yellow and green, it is possible to make the plate react to these rays. Draper's law is therefore obeyed. Chemical reactions caused by light are of many types, but may be divided into reversible and irreversible. The former type of reaction occurs only so long as the light acts (the change from CO- to oxy-hæmoglobin may be given as an example), while the latter type remains in the final state that has been reached (the changes in a photographic plate may be given as an example). There is, further, another and more complicated type which, when once started by an incident beam of light, goes on automatically with an evolution of energy until the reaction is completed. These effects of light are probably of great importance in connection with vision, and will therefore receive further consideration later.

2. EYE MOVEMENTS

ANATOMY AND FUNCTION OF THE EXTRINSIC MUSCLES OF THE EYEBALL

Six external muscles produce rotation of the eyeball:—four recti and two oblique. The recti arise from a fibrous ring attached to the margin of the optic foramen. From the positions they occupy they are called superior, inferior, external and internal. When they contract they cause upward, downward, outward and inward rotation of the eyeball respectively. In the case of the first two muscles there is a turning movement inwards at the same time. Fig. 241 shows the above diagrammatically.

The two oblique muscles, the superior and inferior, are both smaller than the recti. The former arises near the optic foramen, and passes forward to the upper and inner side of the orbit, forming on its way a round tendon. It here passes through a narrow fibrous ring, and then turns downwards and backwards under the superior

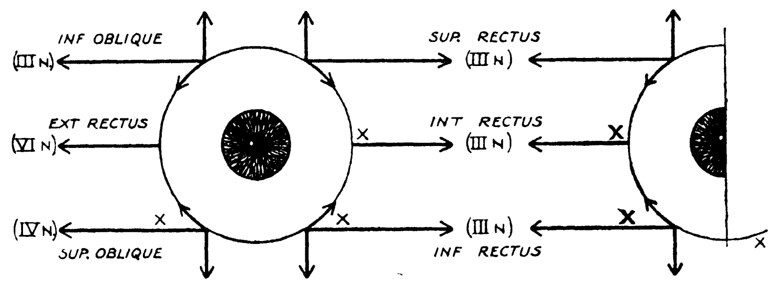

FIG. 241. Diagram showing the Directions in which the different Extrinsic Eye Muscles rotate the Eyeball. x = *crossed connection.*

rectus and becomes attached to the eyeball. The inferior oblique arises from the nasal side of the orbit, just within its lower margin. It passes outwards and backwards beneath the inferior rectus to become attached to the eyeball nearly opposite to the attachment of the superior oblique. On contraction of the superior oblique the upper side of the eye is rotated towards the nose; at the same time the pupil is directed slightly downwards and outwards. The inferior oblique also causes rotation about the visual axes, but in the opposite direction; it at the same time produces upward and outward movement of the pupil.

On tilting the head suddenly about a transverse axis, it is found that the eyes rotate in the opposite direction, so that in fact the image formed on the retina shall still keep in the same apparent meridian. This rotation is called compensatory, and is largely effected by the oblique muscles.

CO-ORDINATED MOVEMENTS OF THE EYES

The notable feature of the eye movements is the close association which exists between the muscles of the two eyes. So perfectly has this mechanism been developed that the eyes are able to glance rapidly from place to place without there being any obvious doubling of the images. The eye movements are therefore of such a kind that the image of an object conveys a single impression to consciousness. But objects vary in their distance from the observer, and therefore, beside movements of the eyes in which the visual axes remain parallel, there are also movements in which there is a certain amount of convergence. In the latter case there is usually some associated accommodation of the lens for near objects, and at the same time some contraction of the pupil. By experiments in which prisms are placed in front of the eyes, thus calling for convergence or divergence without accommodation, and by others in which lenses are placed there instead, thus requiring accommodation without change in the angle between the axes, it can readily be shown that the association between the functions of accommodation and convergence is not very rigid. The co-ordinated deviations of the eyes

appear to be much more closely connected. Thus Donders found co-ordinated deviations both in the newly born and in congenitally blind persons. This is probably due to the close anatomical relationship which exists between the nerve centres of the muscles on the two sides.

THE CONTROL OF EYE MOVEMENTS. The efficiency of the eyes as organs of vision depends very largely on the speed and precision with which their axes can be turned in any required direction. These movements are of three kinds : (*a*) Those in which the eye axes move together to right or left, up or down. (*b*) Those in which the eye axes move in opposite ways, the left eye axis to the right and the right eye axis to the left, thus bringing about 'convergence,' and *vice versa* for 'divergence.' (*c*) Those in which the eyes rotate clockwise or anti-clockwise about their axes. All eye movements can be analysed into combinations of these three components. The movements may be brought into operation in two ways: voluntarily, *e.g.* the directing of the gaze from line to line of print, as in reading; and involuntarily, as when the eyes turn instinctively to scrutinise an object which has presented itself to the field of view, or when the eyes follow an object as it moves; this movement of the eyes is called the 'fixation reflex.'

Six muscles are available to cause movements of each eye, viz., the four recti and the two obliques. Briefly, each of the recti moves the eye principally in one direction : for the four recti, these directions are, respectively, out, in, up and in, or down and in. The obliques, at the same time that they direct the gaze up and out (inferior oblique) or down and out (superior oblique), also cause marked rotation of the eye-balls (Fig. 241).

The eye muscles are innervated by the third, fourth, and sixth cranial nerves, which are derived from nerve cells found in the grey matter of the mid-brain which lies just ventral to the central canal (or Sylvian aqueduct), at the level of the superior corpora quadrigemina. Fig. 242 shows approxi-

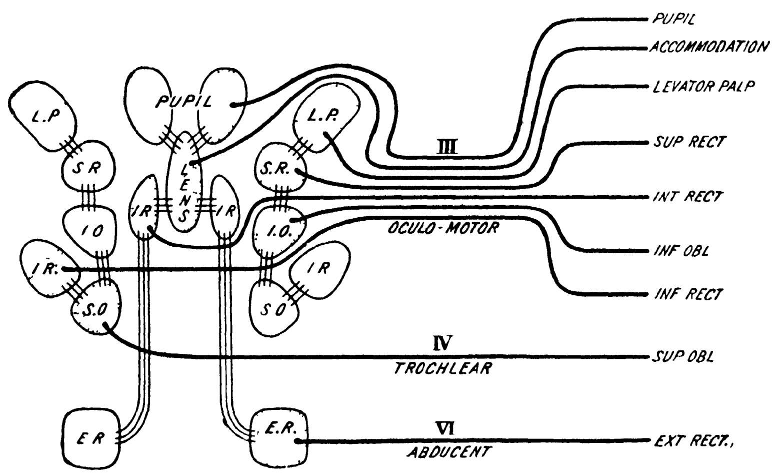

FIG. 242. Diagram to show Relationships of different Points of Oculo-motor Nuclei, and the principal connections between them.

mately the positions of the various collections of nerve cells which innervate the different muscles. It will be seen that the third or oculo-motor nerve supplies all the extrinsic eye muscles except three, viz. : Tenon's capsule, which is supplied by the sympathetic ; the superior oblique, which is supplied by the fourth nerve ; and the external rectus, which is supplied by the sixth

nerve. Further, while most of its nuclei supply muscles on the same side, three are found to go to muscles on the opposite side, namely, the internal and inferior recti (third nerve) and the superior oblique (fourth nerve). Of the many bundles of association fibres which connect these different nuclei, the following may be mentioned as being of special importance: (1) From the nucleus of the external rectus of one side through the posterior longitudinal fasciculus to the nucleus of the internal rectus of the other; thus allowing conjugate deviation of the eyes. (2) Between the nuclei (*a*) for the sphincter pupillæ; (*b*) for the mechanism for accommodation; and (*c*) for the internal rectus; thus co-ordinating the adjustments required for near vision, namely, convergence, accommodation for near objects, and reduced pupil diameter. (3) Between the nuclei of the superior recti muscles of the two eyes; thus causing symmetrical upward deviation. (4) Between the nuclei of the inferior recti of the two eyes for similar reasons. (5) Between the nuclei of the superior oblique of one eye and the inferior oblique of the other; thus permitting conjugate rotation of the eyes. (6) Between the nuclei of the superior rectus and the inferior oblique of the same eye; thus permitting the deviation caused by the one to be corrected by the other. (7) Between the nucleus of the inferior rectus and that of the superior oblique of the same eye for a similar reason. (8) Between the nucleus of the superior rectus and that of the levator palpebræ of the same eye; this association permits simultaneous raising of the eyelid with the upward deviation of the eyes, thus preventing any restriction of vision.

Besides these central connections between the muscles producing like or associated action there are others equally important effected in the brain between those centres which control antagonistic muscles. Sherrington showed that, as the muscle on one side of a limb contracts, its antagonist at the same time relaxes, so as to allow the movement to take place smoothly and without waste of energy. This is called 'reciprocal innervation.' The eye muscles show the phenomenon very well. If the right frontal cortex be stimulated, the eyes perform co-ordinate deviation to the left. If now all the muscles of the right eye except the external rectus are divided, it is found that on repeating the stimulation this eye still moves in co-ordination as far as the middle line, through the relaxation of the external rectus muscle.

The orbicularis palpebrarum is also supplied from the third nerve nucleus, for in lesions of this nucleus paralysis of this muscle is found. The fibres innervating it probably travel all the way with the seventh nerve.

CAUSES AND DIAGNOSIS OF STRABISMUS

Squint or strabismus may be caused by a number of conditions: (1) by congenital abnormality; (2) by interference with the proper rotation of the eyeball; (3) by injury to one of the external eye muscles; (4) by injury to or stimulation of one of the nerves supplying these muscles; (5) by the presence of certain errors of refraction. With regard to nerve injury the following description may be given. Injury to the third nerve causes (*a*) drooping of the upper lid owing to paralysis of the levator palpebræ; (*b*) external strabismus from paralysis of the upper, inner and lower recti and the unopposed action of the external rectus; (*c*) rotation of the eye about its visual axis from paralysis of the inferior oblique and therefore unopposed action of the superior; (*d*) dilatation of the pupil from paralysis of its sphincter and the unopposed action of the dilator fibres which are innervated by the sympathetic; (*e*) loss of the power of accommodation from paralysis of the ciliary muscle; (*f*) exophthalmos or protrusion of the eye, caused by the paralysis of so many of its muscles and the unopposed action of the smooth muscle fibres in Tenon's capsule. Owing to the fact that for a considerable portion of its course the third nerve lies beside the fourth, fifth and sixth nerves, there is usually also some associated symptoms of paralysis in the structures which these nerves

supply. Injury to the fourth nerve causes paralysis of the superior oblique, which shows itself by defective movements in a downward and outward direction. Injury to the sixth nerve causes internal strabismus owing to paralysis of the external rectus. This frequently occurs when tumours, hæmorrhage, inflammation or injuries involve the base of the brain. Experimental stimulation of these nerves causes contraction of the muscles supplied, and does not require specific description.

DIPLOPIA is a characteristic feature of a strabismus. Objects appear double to the patient unless or until he has acquired the ability to suppress the images presented to one (usually the weaker) eye. This suppression gives relief from the diplopia, but involves the risk of the loss of function of the unused (suppressed) eye. An eye that has suffered in this way is said to be amblyopic.

Diplopia is also observed when an individual is under the influence of certain drugs, *e.g.* alcohol. It is also seen sometimes when the spherical curvature of the cornea is disturbed by injury or disease, *e.g.* corneal ulcer. In these cases the injured eye sees a double image even when the sound eye is closed; in fact it may see more than two, the condition being called polyopia.

VOLUNTARY EYE MOVEMENTS. The impulses for these originate in the Betz cells of that part of the voluntary motor area which has its centre about two-thirds down, and somewhat in front of the precentral (Rolandic) strip of cortex. The axons from these cells pass downwards and inwards to form part of the angle (or genu = knee) of the internal capsule. The fibres now descend vertically with the rest of the pyramidal fibres till they reach the level of the superior colliculi where, crossing to the opposite side (as all the other pyramidal Betz cell fibres cross lower down) they end in relation to the third, fourth, and sixth nerve nuclei, either directly or by means of short intervening relay fibres. By this crossing of the fibres the cortex becomes connected with the nerve nuclei of the opposite side. Now the right eye is turned towards the left by fibres which originate in the left third nerve nucleus (see Fig. 242). The left eye is turned towards the left by fibres which originate in the left sixth nerve nucleus. Both these nuclei are controlled by impulses which have originated in the right cerebral cortex. In consequence, impulses from the right cortex turn both eyes to the left. But the crossing of the other voluntary motor fibres causes the cortex on the right side to control the left side of the body, *e.g.* the left arm and hand. The turning of the eyes to the left is therefore very appropriate, since it will cause the gaze to be turned towards the side on which other activities may be in progress.

THE FIXATION REFLEX. Fixation is the retention of the gaze of the two eyes on an external object. It is effected by such adjustments of the external eye muscles that an image of the external object remains on the foveæ of the two eyes. These adjustments are carried out with extreme precision and dispatch. The reflex develops at a very early age, since babies a few days old perform fixation perfectly. Two sets of paths are involved in the reflex: (*a*) Those from retinæ to calcarine cortex *via* the optic radiation; (*b*) those from the calcarine cortex to the superior corpora quadrigemina (colliculi), probably on the opposite side, and from them to the third, fourth and sixth nerve nuclei.

The mode of action of this reflex is probably as follows: Suppose an object, *e.g.* an electric lamp, to be voluntarily fixated, the centres of its images will fall on the foveæ of both eyes and the stimulus produced by it will fall partly on the right and partly on the left halves of both retinæ. Both right and left calcarine cortices will therefore receive impulses. From these fresh impulses will travel to both right and left sets of third, fourth and sixth nerve nuclei, probably *via* the superior corpora quadrigemina of both sides. The eyes will not be moved since the nerve nuclei on one side would produce a movement which is opposed by that of the nerve nuclei on the other side.

Suppose now that the lamp is moved a little to the right. The images produced by the eye media on the retinæ will move to the left and the left-hand halves of the retinæ of both eyes will, as a result, be more strongly stimulated than the right-hand halves. In consequence, the impulses reaching the left-hand calcarine cortex will be more powerful than those reaching the right. Stronger impulses will therefore pass down to the superior corpora quadrigemina (colliculi) and so to the third, fourth and sixth nerve nuclei of the opposite side, that is of the right side. In consequence, the eyes will be turned towards the right, and this will cause the centres of the images of the lamp to fall once more on the foveæ. In some such way as this we may picture fixation being preserved.

Ordinarily fixation is under voluntary control. When this control becomes defective, fixation is still effected, but the gaze 'snaps on to' an external object and remains fixed to it. With a great effort the gaze is turned away from this object only to snap on to another. The use of the eyes, *e.g.* in reading, thus becomes impossible, since the gaze cannot be steered along the line of type.

MINUTE ANATOMY OF THE EYE. THE CORNEA has a radius of nearly 8 mm. and a diameter of 11 mm. It is composed of the following five layers: (1) Stratified epithelium. (2) The anterior elastic lamina of Bowman. (3) Substantia propria. (4) The posterior elastic lamina of Descemet. (5) A layer of epithelium.

THE IRIS consists of three layers: (1) the epithelium, continuous with that on the posterior surface of the cornea; (2) the stroma of the iris, which consists of connective tissue (especially elastic fibres), two thin sheets of muscle, some pigment cells, vessels and nerves; (3) the pigmented layer continuous with the retina.

It should be noted that the posterior elastic lamina of Descemet in the cornea, after its division into three parts, forms by its posterior portion the ligamentum pectinatum iridis, by which the iris gains attachment to the sclero-corneal junction.

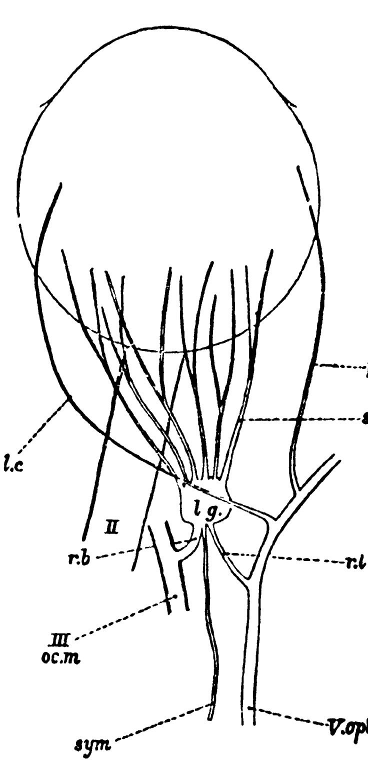

FIG. 243. Nerve Supply to the Eyeball. (After FOSTER.)
l.g. Lenticular ganglion with its three roots, viz:
r.b. Radix brevis or short root.
r.l. Radix longus or long root.
sym. Sympathetic root.
V. opth. Ophthalmic division of *V.* nerve.
III oc.m. Oculo-motor nerve.
II. Optic nerve.
l.c. Long ciliary nerves.
s.c. Short ciliary nerves.

THE FUNCTIONS OF THE IRIS. The iris contains two layers of unstriated muscle fibres; the anterior, which is circularly arranged, so that by its contraction it acts as a sphincter; while the posterior is arranged radially, stretching from the attachment of the iris to the rim of the pupil, so that by its contraction it causes the pupil to open. Because of the numerous pigment cells which it contains the iris is opaque to light. Contraction of the pupil thus causes the following effects: (1) reduction in the amount of light entering the eye, so that an image of less intensity is formed on the retina; (2) the use of the more central zones of the lens system only. The

advantage of this lies in the fact that, as will be described later, the more peripheral zones suffer from errors of refraction to a much greater degree than do the central ones : the contraction of the pupil therefore improves the definition of the image ; (3) an increase in the depth of focus of the eye, which is of great value for near vision. The way that depth of focus is obtained will be described later (see page 411).

Contraction of the Pupil occurs under the following circumstances :

(1) If the third cranial nerve or its nucleus is stimulated electrically, or if the sympathetic nerve fibres to the eye are cut.

(2) When light falls on the retina. This movement, which is known as the 'light reflex,' is determined by a contraction of the sphincter pupillæ, together with a relaxation of the dilator muscle. The contraction ensues within a period of 0·04 to 0·05 sec. after the moment at which the light has access to the retina, and attains its maximum within 0·1 sec. In man as well as in other animals which have binocular vision, and in which there is a partial decussation of the fibres of the optic nerves in the optic chiasma, the reflex is bilateral, *i.e.* light falling into one eye causes simultaneous contraction of both pupils. In the higher animals this reaction of the pupil to light demands the integrity of the nervous paths between the eye and the brain ; but in many of the lower animals, *e.g.* in the frog and eel, the reflex nervous mechanism is aided by a local sensibility of the iris to light. In these animals the contraction of the pupil in response to illumination takes place even in the excised eye, and seems to be determined by a direct stimulation of the pigmented contractile fibres of the sphincter pupillæ by means of the light.

The effect of light on the pupil varies considerably according to the condition of adaptation of the eye. The diameter of the pupil is maximal when the eye has been in the dark for some time and may amount then to 7·3 to 8 mm. In one experiment, on exposing the eye to a feeble light, *e.g.* 1·6 candles at a moderate distance, the pupil diminished in size to 6·3 mm. ; with an illumination of 50 to 100 candles the size of the pupil was 3·7 mm., and with 500 to 1000 candles, 3·3 mm. This effect was obtained by a *rapid* change in the illumination of the eye. When the alteration is sudden, the amount of contraction was found by Haycraft to be equal to the logarithm of the intensity of the light. When, however, the alteration is so gradual that the retina can become adapted to the change as it proceeds, then little or no change in the size of the pupil occurs; and when the illumination, which has at first caused a maximal constriction of the pupil, is continued, the pupil gradually relaxes with the adaptation of the retina to light. This relaxation occurs within three or four minutes after exposure to light has taken place. The same influence of adaptation will be observed if two individuals are brought into a moderately lighted room, one from bright daylight and the other from a dark room. The pupils of the first will dilate widely, while those of the second will constrict. In each case the change will pass off regularly, so that at the end of five or ten minutes there will be no difference observable between the eyes of the two persons.

(3) When vision is directed to a near object, the processes of accommodation of the lens, and of convergence of the visual axes, which result, are associated with contraction of the pupil. The sharpness of vision is thereby improved, together with an increase in the depth of focus, a result very beneficial for the close examination of detail. Since it is possible by experiment to cause accommodation without convergence and *vice versâ,* we may ascertain which function is the more closely associated

with the pupil mechanism. The evidence appears to be in favour of convergence.

(4) In sleep the pupils are always contracted. This behaviour may enable us to distinguish feigned from real sleep. This contraction of the pupils, in spite of the fact that no light is entering the eyes, has been held to be caused by association with the upward and inward direction of the eye axes which was said to be found in sleep. There now appears to be irrefutable evidence that the eyes during sleep may occupy any position; another explanation of the constricted pupils must therefore be found.

(5) Contraction of the pupils is a marked effect of certain drugs such as morphine, which acts by stimulation of the third nerve nucleus; other drugs, *e.g.* pilocarpine, muscarine and eserine, facilitate the action of acetyl choline formed at the 3rd nerve endings in the sphincter pupillæ muscle.

(6) Constricted pupils are also met with in excitable conditions of the central nervous system, and therefore during the induction of chloroform and ether anæsthesia.

(7) Small pupils, which do not react to light, are also met with in injuries to the spinal cord involving the cervical region and damaging the pathway of the sympathetic dilator fibres of the iris.

(8) Contracted pupils are found to accompany severe pain. This is probably due to the powerful flow of efferent impulses which leave the brain in this condition, affecting the 3rd nerve nucleus which controls the pupil.

(9) The pupil contracts when the aqueous is allowed to escape from the anterior chamber. The cause of this is said to be the dilatation of the vessels of the iris, owing to the fall of the surrounding pressure.

Dilatation of the Pupil occurs under the following circumstances:

(1) If the sympathetic nerve fibres to the eye are stimulated, or if the third cranial nerve fibres are cut.

(2) On removal of a light stimulus from the eyes. If the removal be complete the pupil remains dilated, but if there be any light at all the pupil gradually contracts again as the eye becomes dark adapted.

(3) On accommodation for distant vision because the associated reflex stimulation of the pupillo-motor centre with accommodation is no longer called into play.

(4) Reflex dilatation of the pupil can be excited by the stimulation of any sensory nerve. This may be due to some of the afferent impulses reaching the cilio-spinal sympathetic nerve centre in the cord.

(5) The pupils are frequently found to dilate in such emotional states as fear, anxiety, exhaustion and dyspnœa, and also at the moment of death.

(6) Dilatation is also found to accompany extreme exhaustion of the central nervous system, when the activity of all nerve centres is low, such as in deep chloroform anæsthesia, and in the coma produced by alcohol poisoning. Many drugs such as atropine and homatropine, cause dilatation by paralysing the endings of the 3rd nerve. Others, such as cocaine and adrenaline, dilate by stimulating the endings of the sympathetic nerves.

(7) Dilated pupils inactive to light are found in injuries of the 3rd nerve or its nucleus.

(8) Dilated pupils are also found when the intraocular pressure is abnormally high, as in glaucoma. This appears to be due to constriction of the vessels of the iris owing to the high external pressure to which they are subjected.

(9) Dilated pupils inactive to light are found in compression and severe concussion of the brain. This is probably due to the abolition of the normal nervous impulses to the muscles, so that the pupil dilates under the influence of its radial elastic fibres.

(10) Dilated pupils are found to accompany hyperactivity of the suprarenal glands, owing to the presence of considerable amounts of adrenaline in the blood. This occurs for example in fear and in oxygen want, dilated pupils being one of the characteristic signs of those conditions.

(11) The pupils are found to dilate reflexly on stimulating the central end of the splanchnic nerve (McSwiney). The impulses enter the spinal cord by the posterior roots as high as the 3rd thoracic segment. They reach the 3rd nucleus and cause inhibition there.

3. THE NOURISHMENT AND PROTECTION OF THE EYE

Anatomy of the Lids. Closing the orbit in front, and in close relationship to the eyes, are the lids or palpebræ. The upper, which is the larger and the more movable, is provided with a special muscle, the levator palpebræ superioris. This is supplied by a branch of the oculo-motor (3rd) nerve. The two lids meet at an angle on both sides, forming the inner and outer canthi. They are stiffened by two plates of dense fibrous tissue, parallel to their edges, which are called tarsi. Near these and embedded in the substance of the lids are two sets of glands, the Meibomian glands and those of Moll. These secrete a greasy material which spreads over the lids. Superficial to these structures, but under the skin, is a ring of muscle fibres which is common to both lids, the orbicularis palpebrarum, innervated by the 7th nerve. Its contraction closes the lids. Lining the inner surfaces of the eyelids is a thin layer of mucous membrane, the conjunctiva, which is reflected on to the front of the eye, and is continuous over the cornea as the anterior epithelial layer.

Closure of the Lids occurs: (1) during sleep; (2) if a very bright light enters the eyes; (3) on the sudden approach of some foreign body; (4) on contact of a foreign body with the lashes; (5) on irritation of the cornea or conjunctiva, *e.g.* by touching (the *conjunctival reflex*); (6) in sneezing; (7) in order to renew the fluid film on the cornea and conjunctiva. The reflex closure of the lids is therefore a very important function in affording protection to the eyes. The reflex apparently can be initiated by the stimulation of any of the branches of the ophthalmic (1st) division of the 5th (trigeminal) nerve. From the nucleus of this nerve in the pons Varolii, fresh fibres take the impulses, it is believed, to the 3rd nerve nuclei of both sides. The fibres leave the 3rd nerve, however, and join the 7th (facial) nerve, from which they pass to the orbicularis palpebrarum. The conjunctival reflex is one of the last to be abolished by anæsthetics and is therefore used as a convenient test.

The conjunctivæ and the cornea are kept in a moist condition by the tears, which are secreted by the lachrymal gland, situated in the upper and outer part of the orbit. This is a small acino-tubular gland, in microscopic structure similar to the parotid. Its secretion issues through several ducts, the mucous linings of which are continuous with that of the conjunctiva. Normally the secretion is just sufficient to keep the surfaces of the lids and cornea moist, the production keeping pace with the evaporation. Under certain circumstances there is excess, and tears are produced.

Tear Fluid consists chemically of an aqueous solution of sodium chloride and bicarbonate containing mucus, albumin and *débris*. Its functions are to keep the surfaces of the conjunctiva and cornea moist, and to remove foreign bodies and organisms. The secretion of tears is increased (1) by

irritants and foreign bodies coming in contact with the cornea, conjunctiva or lids; (2) by irritation of the nasal mucous membrane; (3) by powerful illumination of the eyes; (4) by the incidence on the eye of infra-red (heat) or ultra-violet (actinic) rays; (5) under the influence of emotion. When excessive tear formation occurs, the fluid either escapes over the front of the lids or is drained away through the lachrymal duct into the nasal sinus. Three theories have been advanced to explain the latter: (1) syphoning, owing to the mouths of the ducts being at a higher level

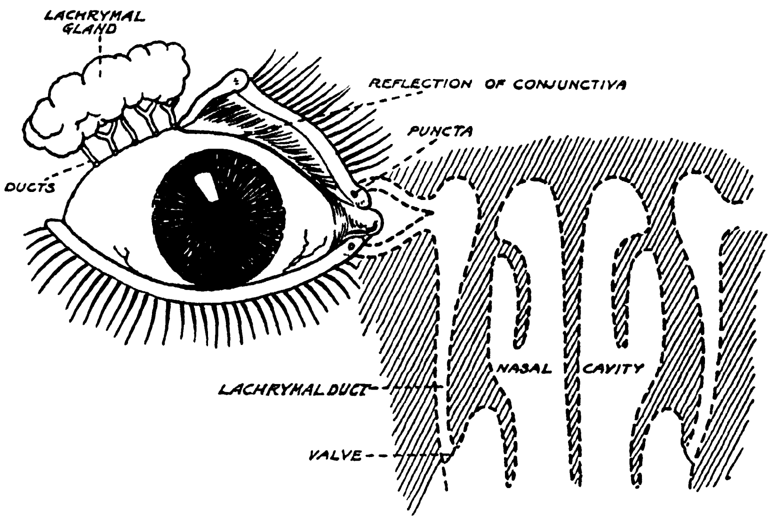

FIG. 214. Diagram to show Origin and Fate of Tear Fluid.

than their exit into the nose; (2) capillarity, owing to the tendency of the liquid to flow into the ducts through surface tension; (3) active removal by the act of blinking. It is not at the present time definitely known how this occurs. Some say that on closing the eye the internal palpebral ligament tends to be pulled on, and that this dilates and fills the lachrymal sac; others that the sac fills automatically through having been previously emptied by the contraction of Horner's muscle. It is possible that both processes occur.

The eyes of some mammals and fish and nearly all birds are provided with a nictitating membrane, a semi-transparent shutter which can be brought over the surface of the cornea. It is supplied with plain muscle fibres innervated by the sympathetic. Stimulation of the sympathetic causes contraction of these fibres, resulting in retraction of the nictitating membrane, and so providing a ready means of estimating the degree of sympathetic activity in laboratory work.

NUTRITION OF THE EYE

The eyeball is richly supplied with blood vessels, which form numerous anastomoses. Among these may be mentioned the arteries of the optic nerve sheath, the long and short posterior ciliary arteries, the anterior ciliary arteries, which are branches from the muscular vessels, and the conjunctival arteries. These pierce the sclera to ramify freely in the choroid and in the ciliary bodies. The iris is supplied by two concentric vessels, the circulus major and the circulus minor. Between the two pass a number of radial

fibres. The retina has a separate blood supply through the central artery of the optic nerve. Other structures, notably the transparent optical media of the eye, have no direct blood supply and therefore depend for their nutrition on diffusion from the aqueous humour. This fluid is formed principally by the ciliary bodies.

AQUEOUS HUMOUR. In chemical composition this fluid consists of water containing salts, traces of albumin and globulin, and a reducing sugar; it is probably freely oxygenated. It has very nearly the same osmotic pressure as the blood (Duke-Elder). The fluid after secretion leaves the eye in one of three ways: (1) By travelling through the pupil into the anterior chamber of the eye and then through the spaces of Fontana at the edges of the iris (the so-called filtration angle) into the canal of Schlemm and thus into the ciliary veins. (2) Through the crypts in the anterior surface of the iris into the veins of that structure. (3) Between the suspensory ligaments of the lens to the anterior surface of the vitreous, then down the hyaloid canal to the papilla of the optic nerve, and thus out *viâ* the lymphatics of the nerve sheath or the retinal vessels. But whatever the fate of the liquid may be, it is certain that the amount secreted must be the same as that which leaves, because otherwise there would be a variation in the intraocular tension. Insufficient pressure will tend to disturb the correct relationship between the internal structures of the eye, and at the same time will prevent the proper action of the ciliary muscle in causing accommodation, because the suspensory ligaments of the lens will already be relaxed. Too great a tension on the other hand will interfere with the proper blood supply to the eye, and will prevent accommodation because the tension in the choroid will be too great for the ciliary muscles to overcome (see page 408). It is therefore important that there should be a proper control of the intraocular pressure. Experiments by Henderson and Starling, in which the intraocular pressure was determined by a null

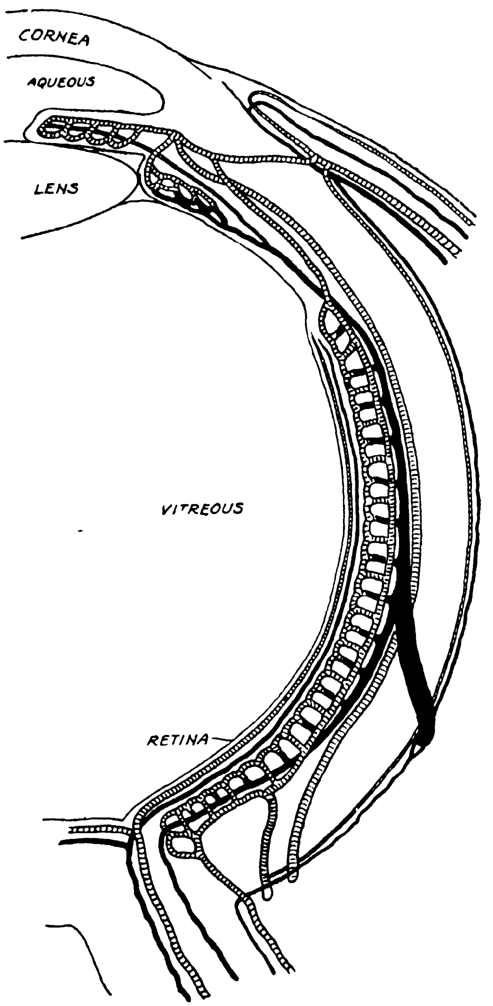

FIG. 245. Diagram to show the Blood Supply of the Eyeball. Arteries 'lined,' veins 'black.'

method (Fig. 247), showed that such a mechanism exists, because as the arterial pressure increased that in the eyeball increased much less.

Whereas the arterial pressure varied between 70 and 180 mm. (*i.e.* a difference of 110 mm.), the intraocular pressure was found to vary between

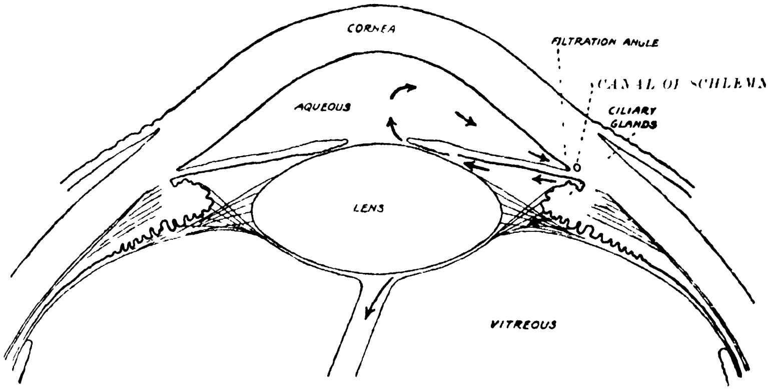

FIG 246. Diagram showing Origin and Fate of Aqueous Humour.

23 and 40 mm. (that is by only 17 mm.). The change in intraocular tension is therefore less than one-sixth of that taking place in the blood ; the control mechanism would therefore appear to have very considerable efficiency.

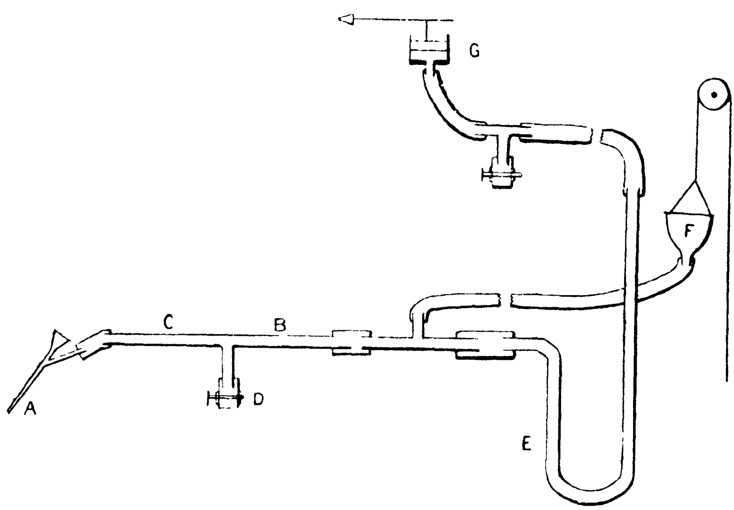

FIG 247. Arrangement of Apparatus for Measurement of Intraocular Pressure. (HENDERSON and STARLING.)

G is a piston recorder for recording graphically the changes in pressure.

GLAUCOMA. The normal intraocular pressure in man is found to be between 25 and 30 mm. of mercury. The tension thus set up in the walls of the eyeball is principally borne by the sclera ; to some extent, however, assistance is rendered by the choroid owing to its elasticity, and by Tenon's capsule owing to the tonic contraction of its smooth muscle fibres (innervated by the sympathetic).

In abnormal conditions the efferent channels may become closed, either from pressure of the lens on the iris (as in hypermetropia) or from the presence of epithelial *débris* in the anterior chamber. The intraocular tension under these circumstances becomes

very high, the disease being known as glaucoma. The principal symptoms of glaucoma are pain and impaired vision. The chief diagnostic signs are a stone-hard eyeball, sluggish, rather dilated pupils, and the retina when examined through the ophthalmoscope is found to show cupping of the optic disc, and vessels which are thin and show pulsation. In treating glaucoma, operative measures to lower the pressure should be taken immediately, because the high pressure interferes with the proper blood supply to the eye. Since all hypermetropes (persons with long sight) have a tendency to suffer from glaucoma, care should be taken against giving drugs such as atropine which cause dilatation of the pupil, since this increases the resistance to the escape of fluid at the filtration angle, and therefore predisposes to an attack of glaucoma.

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4. THE OPTICAL SYSTEM OF THE EYE

The optical system of the eye consists of those structures which together focus an image of external objects on the retina. In the mammalian eye there are four concerned: the cornea, the aqueous humour, the crystalline lens and the vitreous humour. The histology of the cornea has already been considered. The aqueous humour is a structureless liquid. The vitreous

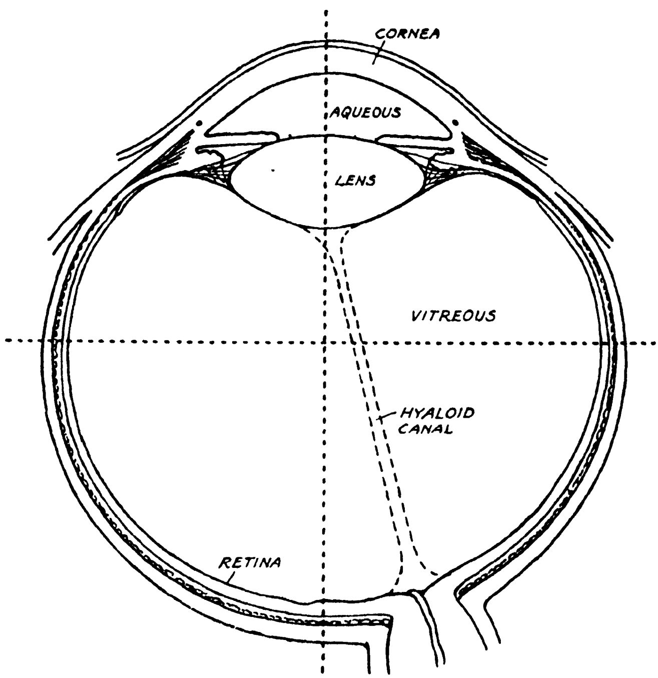

Fig. 248. Transverse Section through Equator of left Eye seen from above.

humour which consists of a collagenous material is enclosed in the hyaloid membrane, and through the middle of it, during fœtal life, runs the hyaloid artery, which goes from the central artery of the retina to the posterior surface of the lens.

The Crystalline Lens is a biconvex transparent elastic body, enclosed in an elastic membrane called the capsule. To the periphery of the capsule are attached the suspensory ligaments of the lens, which are formed by the anterior radial fibres of the thickened portion of the hyaloid membrane (the zonula of Zinn) (Fig. 249). Between the suspensory ligaments are shallow pockets into which the ciliary processes fit closely. In this way the lens is held firmly in position, while at the same time, by the movement of the ciliary processes under the action of the ciliary muscle, the traction can be altered in the suspensory ligaments, thus effecting the change in curvature of the lens, which will be shown later to be necessary for accommodation.

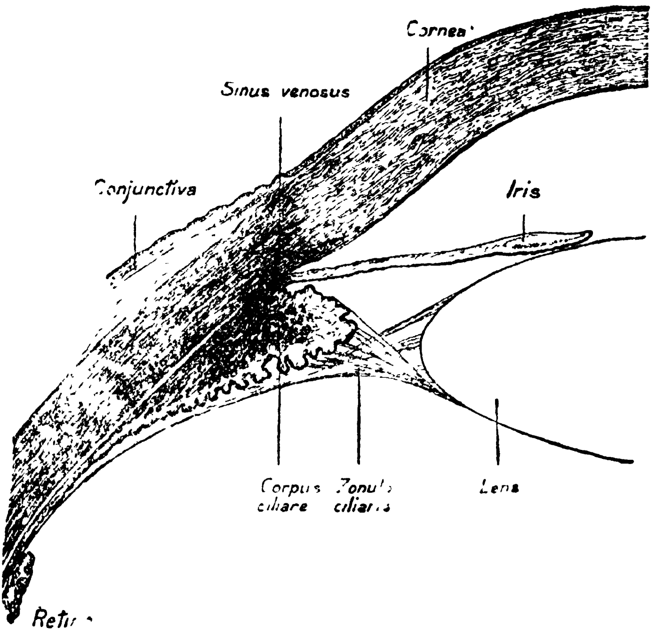

Fig. 249. Section through Anterior Part of Eyeball to show Mode of Suspension of Lens. (After Merkel and Kallius.)

Histologically the lens is composed of a number of radially arranged fibres, each of which is a modified epithelial cell. These fibres are arranged in concentric layers, the more peripheral being soft, nucleated and of low refractive index, while the central form a dense non-nucleated mass of high refractive index, the fibrous layers between having an intermediate structure and index.

Metabolism of the Crystalline Lens. If the crystalline lens be removed from the eye of an animal and placed in a microrespirometer of suitable pattern, it can be shown that oxygen is taken up by the lens and that carbon dioxide is given out. If such a lens is deprived of oxygen, or is exposed to excess of carbon dioxide for some hours, it shows signs of degeneration. The lenses of young animals have a high cystine content, which is associated with an active glutathione oxidation system. As animals get older the cystine content falls, and this may be associated with the decreased rate of metabolism which the lens shows.

Refraction by the Crystalline Lens. Refraction occurs whenever light passes from one medium into another of different optical density. It

is due to the fact that the waves, of which the beam of light is composed, travel more slowly in a dense medium than they do in one of less density. Some of the effects which this produces are shown in the diagram below.

At A, plane waves are seen entering a dense medium at an angle. At B the medium is lens shaped. At C the medium has a plane surface but has a greater density below than above. At D the medium has a plane surface but a greater density at its centre than at its edges. At E the medium is lens shaped as at B, and also varies in density as at D. The very great refracting power of such a structure is well shown. This is the arrangement found in the lens of the eye, in which, therefore, the power of refraction is very much greater than that of an ordinary lens of the same curvature as the lens and of the same refractive index as the average density of its substance. The optical properties of the lens are therefore unique, and it is interesting to find the same (*i e.* even immunologically identical) protein in the lens of different species of animals.

To show the effect which the increasing density of the lens produces, the refractive indices of its parts may be compared with its equivalent R.I. (that is, the refractive index of a glass lens of the same size, shape and focal length). The refractive index of the periphery of the lens is 1·37, and that of the central nucleus 1·41, the mean being about 1·39. But the equivalent density of the lens is found to be 1·42, that is, greater by 0·03 than the mean refractive index of its substance. The lens lies in contact with two transparent media, both of which have an approximate refractive index of 1·34. The power of the lens, if its composition were uniform, would therefore be pro-

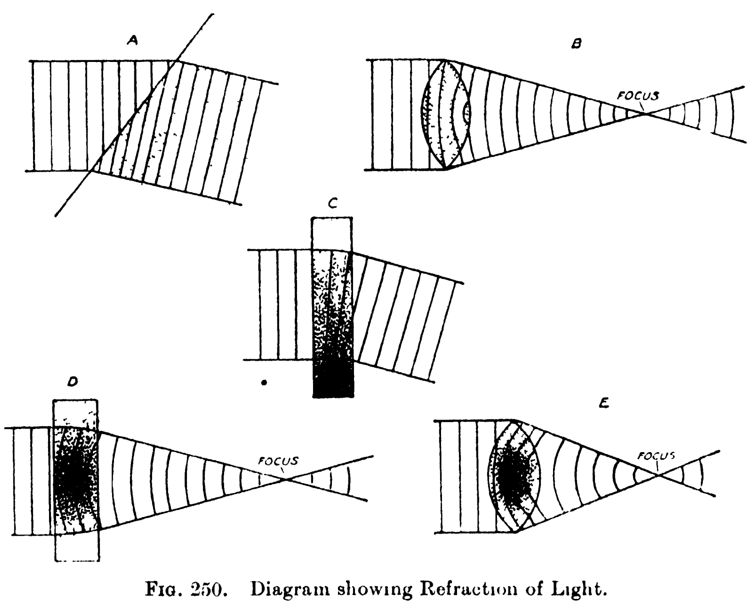

FIG. 250. Diagram showing Refraction of Light.

(A). By an inclined surface.
(B). By a lens.
(C). By a plate of greater density at its lower end than at its upper.
(D). By a plate of greater density at its centre than at its edges.
(E). By a lens of greater density at its centre than at its edges.

portional to the difference between its own mean R.I. (1·39) and that of its surroundings (1·34), that is to 0·05. Owing to its peculiar structure its equivalent R.I. is 1·42, and therefore its power is proportional to the

difference between that and 1·34, that is to 0·08. Owing to its structure the lens has therefore increased in power in the ratio of 0·08 to 0·05. Now

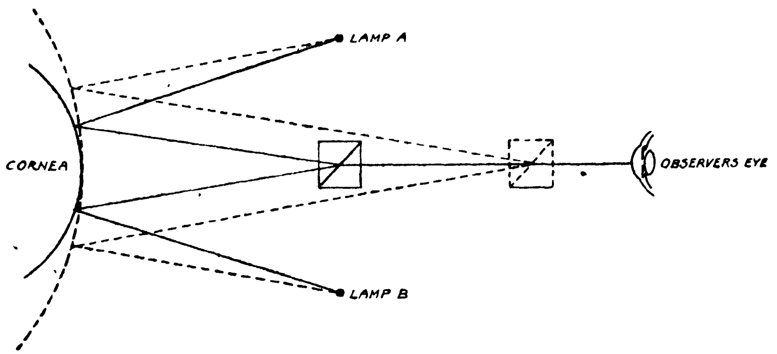

FIG. 251. Diagram to show a Method of Determining the Curvature of the Anterior Surface of the Cornea.

The images of the lamps A and B are caused to coincide by shifting the position of the double image prism. The greater the curvature of the cornea the closer must the double image prism be to the eye.

since the range of accommodation depends, other things being equal, on the power of the lens, we see that the peculiar structure of the lens has nearly doubled its range. The graduation in the densities of the different layers of the lens has a further advantage which will be described later, in that it reduces the spherical aberration of the eye as a whole and also reduces the amount of scattered light within the eyeball.

THE OPTICAL CONSTANTS OF THE EYE. In the case of the crystalline lens, two methods are available for the determination of the radii of curvature of the anterior and posterior surfaces, namely measurements on the excised lens, either in the air or preferably suspended in a fluid of known optical properties, or by estimating the apparent size of the images of an object which are formed by reflection at its surfaces. In order that the latter method shall succeed, a device must be employed for eliminating the effect of chance movements of the eye while under observation. This was first done by Thomas Young, who employed a method used in astronomy, namely that of doubling the image to be measured and then adjusting the lower edge of one image to be in coincidence with the upper edge of the

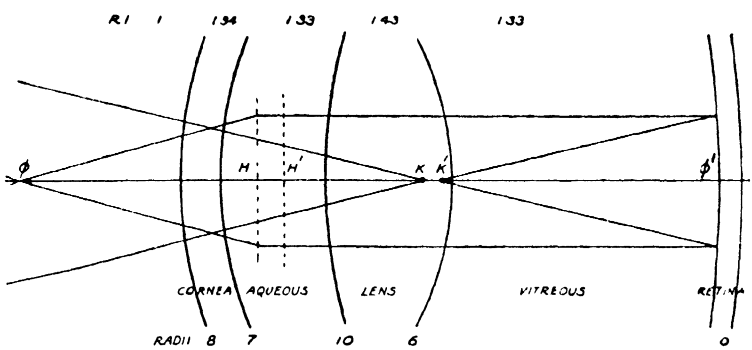

FIG. 252. The Optical System of the Eye shown diagrammatically.

H and H′ principal points. K and K′ nodal points. ϕ and ϕ' anterior and posterior foci.

other. If the eye moved during the determinations, both images moved together and therefore difficulties in adjustment were avoided. In the case of the cornea this method is alone available because only in the living state is the true curvature preserved (Fig. 251). In the case of the lens the determinations are complicated by the fact that the refraction of the cornea has to be allowed for. Further, the images that are seen are neither bright nor sharply defined; but in spite of this, considerable accuracy is attainable. The following are the approximate values given by these methods.

Radius of cornea	8 mm.
Radius of lens, anterior surface	10 ,,
Radius of lens, posterior surface	6 ,,

THE REFRACTIVE INDICES (optical densities) of the eye media are determined on the excised eye by means of the Abbe refractometer. It is found that the cornea and aqueous are so nearly alike that for all practical purposes they may be regarded as one, particularly as the posterior corneal surface has nearly the same centre as the anterior. The refractive indices may therefore be given as follows :—

Refractive index of cornea and aqueous	1·34
Refractive index of lens (equivalent)	1·42
Refractive index of aqueous humour	1·33

THE APPLICATION OF GAUSS' THEOREM. In addition to the above data we require to know the distance between the principal surfaces ; these are found to be :—

Distance from cornea to anterior lens surface	3·6 mm.
Distance from cornea to posterior lens surface	7·6 ,,
Distance from cornea to the retina	22·6 ,,

These values being known, it is possible by calculation to determine the path of any ray through the eye. The problem, however, is made very much simpler by the application of Gauss' theorem, which may be briefly stated as follows. Any system of spherical optical surfaces, the centres of which lie along a straight line ("the axis"), possesses six cardinal points—namely, two principal points H and H′ (Fig. 253), two nodal points K and K′, and two focal points, the anterior ϕ and the posterior ϕ'. Passing through each of the

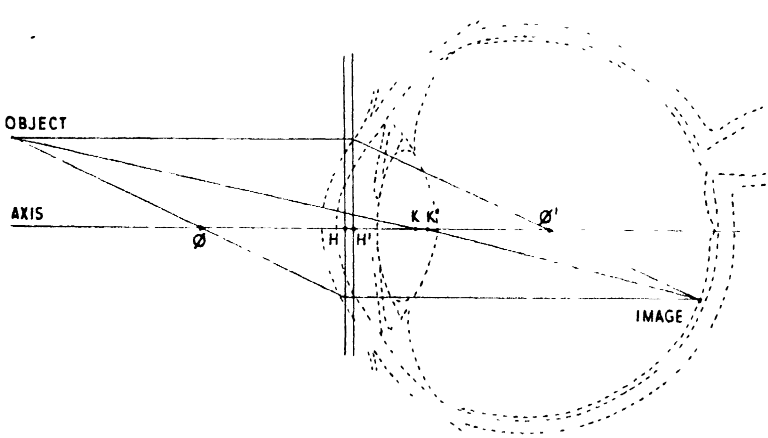

FIG. 253. Diagram showing approximately the positions of the principal planes and nodal points of the eye. The course of rays from a near object passing through the eye to form an image on the retina is shown.

principal points there is a plane called the principal plane, and this is perpendicular to the axis (see vertical lines through H and H′ in Fig. 253). It is found that these points and planes have certain properties which may be summarised as follows :—

(1) Any ray incident on the nearest principal plane and striking it at a distance x from the axis is continued as a ray which leaves the other principal plane at the same distance from the axis. (2) Any ray passing through the first nodal point on its way into the system appears to come from the other on its way out, but its direction is still parallel to the path which it traversed initially. (3) Any ray passing through either focus leaves the lens system on the other side parallel to the axis. It is further found that the distances between some of these points are equal, for example :—$\phi H = K'\phi'$ and $H'\phi' = \phi K$. Also $HH' = KK'$. The position of these cardinal points has been determined in the case of the eye with considerable accuracy; the following approximate values may be given :—

Distance from front of cornea to first principal point H .	1·7 mm.
Distance from first principal point H to second principal point H′	0·3 ,,
Distance from second principal point H′ to first nodal point K	5·0 ,,
Distance from first nodal point K to second nodal point K′ .	0·3 ,,
Distance from first principal point H to anterior focus ϕ .	15·3 ,,
Distance from second nodal point K′ to posterior focus ϕ' .	15·3 ,,
Distance from front of cornea to retina	22·6 ,,

The position of these points being determined, the direction of the rays of light through the eye can be easily obtained, and is shown diagrammatically in Fig. 353.

It is of some interest to know the relative part taken by the various refractive media of the eye in the formation of the image. By far the greater part is performed by the cornea. The following values in mm. and dioptres * may be given :—

	Mm.		Dioptres.
Focal length of the cornea	24	..	42
,, of the lens	44	..	23
,, of the whole eye	15·5	..	65

When opacities form in the lens (cataract) this structure is removed by operation. It is then found that a lens of approximately 10 dioptres has to be worn by the patient in order that he may see distinctly. To this a cylinder has usually to be added to correct astigmatism introduced into the eyeball from the scar of the incision ; and a 4-dioptre spherical lens has to be added when a glass for reading is being prescribed. If the glass lens could be placed inside the eyeball in the exact position occupied by the crystalline lens, it would, as the above Table shows, require a power of 23 dioptres instead of 10 dioptres. When the eye is placed under water, the refraction of the cornea is necessarily abolished because water has approximately the same refractive index. Under these conditions the eye becomes too long-sighted for distinct vision. The eye of the fish has met this difficulty by the provision of a small, nearly spherical lens of very great density. In the fish, therefore, the lens takes the principal part in the refraction of the light in the formation of an image.

Reduced Eye. It is a convenient fact that, owing to the closeness of the two principal and the two nodal points to one another, it is possible

* In the dioptre nomenclature the figure represents the reciprocal of the focal length in metres. Thus 10 dioptres = $\frac{1}{10}$ metre = 100 mm.; and 24 mm. = $\frac{1000}{24}$ = 42 dioptres.

to imagine the media of the eye replaced by a single optical surface without introducing any appreciable error. To this system is given the title 'reduced eye.' Its constants are given in the following Table :—

Radius of surface	5	mm.
Distance of principal point from anterior surface	2·3	,,
Distance of nodal point from anterior surface	7·1	,,
Distance of retina from anterior surface	22·6	,,
Refractive index	1·33	,,
Focal length	15·5	,,

THE ACCOMMODATION OF THE EYE

The above description has been made on the supposition that the rays entering the eye consist of parallel bundles, or in other words that the objects

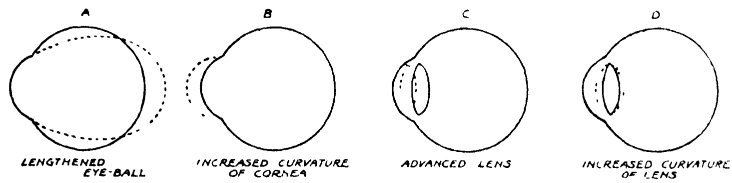

FIG. 254. Methods by which Accommodation of the Refraction of the Eye for Objects at Different Distances could be effected.
(A). By lengthening the eyeball. (C). By moving lens forward.
(B). By increasing curvature of cornea. (D). By increasing curvature of lens

seen are at an infinite distance from the eye. But during near vision, such is by no means the case. If there were no means of varying the focus of the eye, it would not be possible for divergent rays (those coming from near objects) to be brought to a focus on the retina. The mechanism for varying the focus of the eye is called accommodation.

THE THEORIES OF ACCOMMODATION. Of these, three are of historic interest only: (1) That during accommodation the cornea increases in curvature (similar to the bird's eye). This was disproved by Thomas Young, who placed his eye under water, replaced the corneal refraction by a convex lens, and then found the amplitude of accommodation unaffected. (2) That the eye elongates in near vision, thus causing the rays from near objects to focus on the retina (similar to the arrangement in the mollusc Pecten). This also Thomas Young disproved by placing two iron rings which could be clamped together, one in front of and one behind the eyeball. Having very prominent eyes he could do this if the eye being tested were rotated strongly inwards. The "phosphene," or subjective appearance, caused by the pressure of the posterior ring, which extended to the fovea, did not change in appearance during accommodation. He thus found no evidence for an elongation of the eye during accommodation (3) That the lens during near vision advances towards the cornea (similar to the mechanism in the fish's eye). This view was disproved by Tscherning, who calculated that the lens would have to advance nearly 10 mm. in order to give the full amplitude found for the eye, whereas the anterior chamber of the eye is, as we have seen, approximately only 2·6 mm.

It follows that in the mammalian eye the fourth method is employed, viz., that accommodation is effected by increasing the curvature of the lens. As to the way in which this is brought about, there are three theories, those of Helmholtz, Tscherning and Thompson Henderson. The first theory (which is the one most generally accepted) supposes that the lens when removed from the eye is strongly convex and is accommodated for near vision. When in the eye, however, it is caused to become flatter through the traction of the zonula of Zinn (suspensory ligaments) on the edges of its capsule, and is therefore focussed for distance. But when the ciliary muscle contracts, it removes the tension on the zonula and therefore allows the lens to return by its elasticity to its more spherical form. Before describing the rival theories it would be well to examine the principal evidence on which Helmholtz' theory has been based. During near vision,

measurement by means of the ophthalmometer shows that the anterior surface of the lens advances slightly and becomes at the centre of much greater curvature (10 mm. radius for distant vision, to 6 mm. for near). There can thus be no question that the change in the curvature of the lens is responsible for accommodation. The posterior surface is found to change but little; almost the whole range is therefore produced by the anterior surface. The changes found to occur in the lens of an adult twenty-five years old may therefore be summarised as follows :—

	Distance		Near.
Radius of anterior surface, in mm.	10	..	6
Radius of posterior surface, in mm. . .	6	..	5·5
Thickness of lens, in mm.	3·6	..	4
Focus of lens, in mm.	44	..	30
Focus of lens, in dioptres	23	..	33
Range of accommodation, in dioptres . .	10		

If the lens in near vision becomes more spherical owing to the relaxation of the zonula, as Helmholtz supposed, we should expect a lens removed from the eye to be more spherical still, that is, in a state of strong accommodation. Tscherning stated that the changes in the curvature of the lens are much more complex than those given above. During accommodation not only is there, he says, an increase in the curvature near the centre of the lens, but at the same time a decrease in the curvature at the periphery. This view he supported by quoting the careful measurements which Young made by means of his optometer, and which have been confirmed by other observers. There is found to be a zone about 1 4 mm. from the centre of the lens where the curvature does not change appreciably. Inside this zone the curvature increases during accommodation, whereas outside the lens becomes flatter. Tscherning supposed that these changes of curvature are produced by an increase in the tension of the zonula during accommodation, in other words exactly the opposite action to that which Helmholtz supposed to occur. According to Thompson Henderson, the setting up of tension in the suspensory ligaments is due to the pull of the muscles of accommodation, that is, that these muscles are in tension during accommodation to distant vision. In the excised eyeball, therefore, the lens will be adjusted for near vision.

We may summarise the position with regard to these theories as follows :—

According to Helmholtz, the muscles adjust the lens for near vision. According to Tscherning and Thompson Henderson, they adjust the lens for distant vision. The state of the lens in an excised eye and when isolated from the eye is therefore as follows :—

	Helmholtz	Tscherning	Thompson Henderson
Lens in excised eye accommodated for	Distant vision	Near vision	Near vision
Isolated lens accommodated for . .	Near vision	Distant vision	Near vision

Experiments have shown that the choroid moves forward during accommodation in both man and animals; further, Hess has shown that, when full accommodation has been performed, the lens is only loosely supported, so that gravity can act on it and cause it to sink slightly in relationship to the other eye structures. These effects appear to be definitely in favour of Helmholtz' theory, and against those of Tscherning and Thompson Henderson. Additional evidence on this point is provided by the experiments of Hartridge and Yamada (1922).

They obtained the following values in the cat's eye :—

Power of lens in excised eyeball	65 dioptres
Power of isolated lens	80 ,,

The lens in the excised eyeball is therefore in the condition for distant vision, whereas the isolated lens is in the condition for near vision, there being an increase in the power of the lens of 15 dioptres. These results are in favour of Helmholtz' theory and contrary to the theories of Tscherning and Thompson Henderson.

THE MECHANISM OF ACCOMMODATION. The ciliary muscle consists of two separate sets of unstriated muscle fibres : the more superficial set of radial or longitudinal fibres, the deeper of bundles of circular fibres. The former take their origin from the sclero-corneal junction, and are attached to the anterior part of the choroid coat behind the ciliary processes. When these fibres contract, they draw the choroid forward and inward, the ciliary processes tending to occupy a smaller circle. The circular fibres lie in the substance of the base of the ciliary processes, so that, when they contract, they cause the apices of the processes to approach one another. In addition to these two sets of fibres, a third set has been described as meridional. These are, however, part of the radio-longitudinal set from which there does not appear to be much object in differentiating them. The ciliary muscles therefore have a common action, causing the ciliary processes to form a smaller circle. The zonula of Zinn, or suspensory ligament, is formed of a large number of very fine fibres which run from the ciliary processes to the

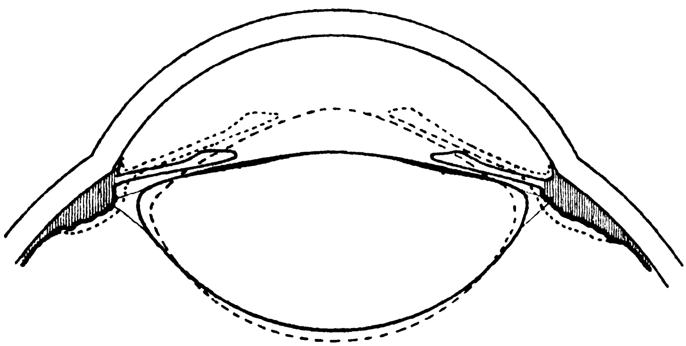

FIG. 255. Diagram to show the Changes in the Position and Shape of the Eye Structures during Accommodation. (After FINCHAM and HELMHOLTZ.)

capsule of the lens. Further, those which arise posteriorly are attached anteriorly and *vice versâ*. When the ciliary muscle is in a state of rest, the tension in the choroid set up by the intraocular pressure causes the ciliary processes to be pulled in an outward and backward direction and therefore puts tension on the lens capsule through the zonula. The lens thus tends to be flattened and accommodated for distance. On contraction of the

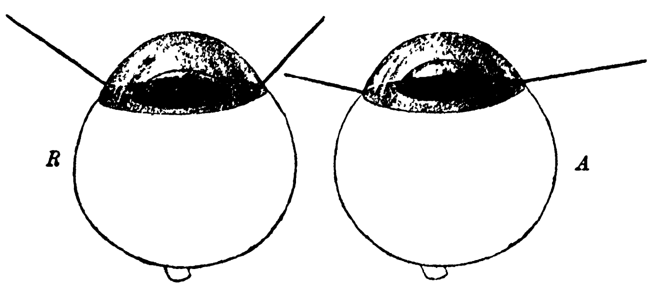

FIG. 256. Accommodation in the Cat's Eye. (BEER.)

R. Distance. *A*. For Near Vision.

Two needles have been passed through the edge of cornea into the ciliary bodies, to show forward movement of the latter during accommodation.

ciliary muscle the tension in the choroid is opposed and the ciliary processes approximated (Fig. 256). The zonula thus becomes slack, and the tension of the lens capsule decreases, allowing the lens to take up its more natural spherical shape, and thus to focus the rays from nearer objects.

Fincham has pointed out the important part played by the variations in thickness of the capsule of the crystalline lens. This is very thin at the front surface near the centre, but very thick 2 or 3 mm. peripheral to the centre. The result of this is that the tension in the capsule causes the central part of the lens where the capsule is thin to become more highly curved, whereas it causes the more peripheral part of the lens where the capsule is thick to become flattened. These effects of the capsule are shown in Fig. 255 for both the unaccommodated and the accommodated lens.

INNERVATION OF MECHANISM OF ACCOMMODATION. The ciliary muscle is innervated through the 3rd nerve, its nucleus being situated near the mid line under those of the pupils. Owing to the close association of the nuclei on the two sides, it is impossible to cause accommodation of the eyes separately. From this nucleus the fibres travel down with the rest of the nerve through the outer wall of the cavernous sinus, and when they reach the orbital cavity are given off to the ciliary (lenticular) ganglion, where they anastomose with nerve cells, the processes of which then proceed through the short ciliary nerves to the eyeball.

The position of the higher centre connected with the nuclei concerned in accommodation is not definitely known; it is believed to be in the occipital cortex. But since it is possible to carry out willed changes of focus as well as subconscious ones there must be connections with other parts of the brain as well.

THE AMPLITUDE OF ACCOMMODATION in the emmetropic (normal) eye is measured by ascertaining the nearest point from the eye at which perfect vision can be obtained. Since it is possible that the eyes, when examined separately, can focus nearer objects than they can when used together (owing to the limitation in the power of convergence), one of the eyes should be closed when making the determination. In the ametropic (abnormal) eye it is necessary to determine the far point as well as the near, since the former may not be at infinity as it is in the emmetropic eye. But other difficulties are encountered because, in the case of hypermetropic (long-sighted) eyes, an object placed at infinity still requires some accommodation in order to focus it. Lastly there comes the personal equation of the patient, since it is found that, even when apparently fully relaxed, the instillation of atropine usually causes some further relaxation of the accommodation; so in the same way the instillation of eserine is usually followed by a definite increase in the accommodation over that which can be voluntarily exerted. To obtain the maximum amplitude of the accommodation these drugs should therefore be used. Where a comparative and relatively approximate value is alone required they may be omitted. Of the many methods that may be employed, probably the simplest is by the use of the set of trial lenses, which vary in their curvature by small uniform amounts from strongly convex (plus) to strongly concave (minus). The test object consists of a pin placed vertically in a board at any fixed distance from the eye, a white surface being arranged behind it. A pair of spectacles to hold the trial lenses is placed before the eyes, and in them are inserted two metal plates with two vertical slits in them, so that each eye in turn may look at the test object through the slits in the plate opposite it. To determine the *near point*, minus (concave) lenses of gradually increasing power are placed before one of the eyes, the other being closed, until one is found that just causes the image of the pin to appear double. The power of the next weaker lens is therefore taken to be the correct one. To determine the *far point*, plus (convex) lenses of increasing power are tried in a similar manner, and that one taken which just does not cause appreciable doubling. The difference between the power of the two lenses found in this way gives in dioptres the value of the amplitude of the accommodation. Care should be specially taken to see that the value found for the concave lens has the minus sign placed in front of it. Thus if it was found that the far point was reached by the use of a convex lens of 2·25 dioptres, and that the near point required a concave one of 7·5 D., then the amplitude is not 5·25 D. but 9·75 D. Careful measurements made in the above manner show that there is for different ages an average range of accommodation. In youth the amplitude is large, but it decreases uniformly to old age, and this decrease is called *presbyopia* (old-sight). The amplitude found as a rule at different ages is approximately constant and is given in the Table below.

Age in Years	Accommodation in Dioptres.
10	13·8
15	12·6
20	11·5
25	10·2
30	8·9
35	7·3
40	5·8
45	3·7
50	2·0
55	1·3
60	1·1

This gradual reduction in the amplitude of the accommodation is caused by the hardening of the crystalline lens, which with the advance of years robs it of the elasticity of youth. As a rule the change goes on unheeded till the time comes when the near point of vision has receded so far from the eye that the book has to be held at a distance, in order to be focussed clearly. Then it is found that the print, particularly if it is fine, becomes unreadable, and convex glasses have to be worn. It is at that time that presbyopia may be said to begin, usually at an age of 45 to 50 in emmetropia. In hypermetropia, presbyopia shows itself earlier than normal, and in myopia (short-sight) later. The reason for these differences will be described when dealing with these conditions.

THE EFFECT OF DRUGS on the accommodation has already been alluded to. In ophthalmic practice three are used in order to paralyse the ciliary muscle, namely atropine (one-half per cent. solution), homatropine (2 per cent.) and scopolamine (one-fifth per cent.). One of these drugs is placed within the conjunctival sac, and from here it slowly travels, probably by diffusion *viâ* cornea and aqueous, to the ciliary and iris muscles, both of which are paralysed. This fact is of the greatest importance, for if this path did not exist the use of these valuable drugs would hardly be possible. Contraction of the ciliary muscles and of the sphincter pupillæ is caused by the drugs pilocarpine and physostigmine (eserine). One of these is often used to counteract the effects of the atropine group.

5. THE REFRACTION OF THE EYE

Since the eye forms an image of external objects by means of its refracting media, it is found to have properties and to suffer from defects similar to those met with in the case of other optical systems. We may therefore treat the eye as if it were an optical instrument, and estimate its efficiency from that point of view. In the first place, therefore, we must consider what kind of an image it would form if it were a perfect lens system, suffering from no kind of aberration. Our experience of other lens systems, well-nigh perfect, has shown us that the image of a distant point source of light is not a mathematical point, as geometrical optics would have us believe, but is on the contrary a definite pattern of quite considerable size, the shape and dimensions of which can either be calculated from the conditions, or may be seen, measured and photographed by appropriate means. The formation of this pattern is due, not to any defect of the lens, but to the fact that light is a form of wave motion, which exhibits a property called diffraction.

The diffraction pattern formed by any lens system varies directly as the focal length of the system and the wavelength of the light, and inversely as the diameter of the pupil through which the light passes. In the case of any lens system such as the eye, in which the pupil is circular, experiment and calculation are agreed that the image of a point source consists of a series of concentric rings of light, having a bright spot at their centre. The diameter of this spot in the case of the eye is found to be 0·01 mm. with a pupil of 2 mm. diameter. No matter how perfect the eye be as an optical instrument, diffraction sets a limit in this way to the perfection

of the image that can be formed. This should not, however, be thought of as a defect, but as a property, since it is caused by the nature of light itself. In the consideration of the principal optical errors of the normal eye, we have to decide in each case, not only to what extent the defect is present, but whether the defect produces any noticeable change in the diffraction pattern which may affect definition.

DEPTH OF FOCUS, like diffraction, is a property of a lens system and not an aberration. Its origin may be explained as follows:—Suppose objects 100 metres away to be forming sharp images on the retina, then objects at 200 metres will form images which come to a focus slightly in front of the retina, and objects at 50 metres images that are slightly behind. If however the focussed points are only a short distance in front of or behind the retina, the image of a distant point which fell on a single cone would still do so although its distance from the eye had been altered, because the cone has a certain diameter. Depth of focus in the case of the eye is the greatest distance through which a point can be moved and still produce an image which falls exactly on a cone without spreading at all on to neighbouring ones. For example, in the above case the distance moved was from 200 to 50 metres, that is, the depth of focus was 150 metres. Now it is found in the case of any lens system that depth varies with the aperture of the pupil. Thus in the case of the eye the following values are obtained:—

Pupil diameter.	Depth at infinity	Depth at 25 cms.
1 mm.	From inf. to 8 metres	3·2 cm.
2 „	„ „ 16 „	1·6 „
3 „	„ „ 24 „	1·1 „
4 „	„ „ 32 „	0 8 „

We see, therefore, that not only does depth decrease as the aperture of the pupil increases, but that it also decreases as the mean distance of the objects from the eye decreases. Thus with a pupil of 3 mm. the eye, if focussed sharply on objects 24 metres away, would also be in focus for objects at infinity and for objects at 32 metres. Depth of focus is therefore considerable at this distance. But if the eye is working at the distance of 25 cm., depth would be 1 1 cm. only. At a pupil diameter of 1 mm., depth would be increased threefold, and therefore the closure of the pupil, which accompanies accommodation and convergence for near objects, has the valuable property of increasing the depth of focus at the same time. The average reading distance depends on the size of the individual, i e., on the length of the arm, and is about 35 cm. (14 in.) for ordinary adults.

CHROMATIC ABERRATION OF THE EYE. We have seen that white light consists of a number of rays of different wavelength, and that on refraction the short rays are more bent than the long. When, therefore, white light is incident on a lens, the rays of short wavelength come to a focus in front of those of longer wavelength. This difference of focus for rays of different colour is called chromatic difference of focus. Experiment shows that, when such a series of foci are formed by the eye, the accommodation is so adjusted that the rays of greatest intensity (usually yellow rays) form the most sharply focussed image, and the colours of longer and shorter focus form blurred discs of light of relatively low intensity on top of this. Under these conditions it is found that quite well defined images are produced. Thus, with a pupil of 2 mm. diameter, approximately 70 per cent. of the light falls in an area of 0·005 mm. diameter.* Further it may be shown that a lens system such as the eye, which suffers from chromatic aberration, produces an image that is only just appreciably worse than one that is perfectly corrected, when the effects of diffraction are taken into account in both cases. But since the effects of chromatic aberration increase as the pupil enlarges, while those caused by diffraction decrease, it is clear that the larger the pupil the more does chromatic aberration tend to spoil definition. But as this is

* Hartridge. *J. Physiol.*, 1918, **52**, p. 175; and 1922, **57**, p. 52.

accompanied by a decrease in diffraction, the two changes taken together have the effect of leaving the actual definition practically unchanged. This important conclusion will be referred to again more fully in the last section. Beside effects on definition, chromatic aberration causes small bright points of light on a dark ground to form images which are largely composed of yellow rays, and on the other hand small black objects on a bright ground to be purple in colour. The reason for these colours being unnoticed in ordinary circumstances is due to the recognition by the eye of the presence of the complementary colour which forms a fringe round the central point.

Spherical Aberration of the Eye. The employment of spherical surfaces as boundaries for optical media leads to a difference in the position of the foci of rays that have passed through the centre of the lens and those that have passed through the more peripheral parts. The latter usually form a focus nearer to the lens. Since the eye is bounded by nearly spherical curves, it might be assumed that this aberration must be present in this organ. It should be remembered, however, that the crystalline lens has a structure quite different from that found in the lens systems of optical instruments. For the presence of a graduation of optical density, culminating in a nucleus of relatively great curvature, causes rays passing through the centre of the eye to be refracted to a greater extent than more peripheral rays, or, in other words, exactly the opposite effect to that produced by spherical aberration. Measurements on the eyes of different individuals, therefore, show the presence both of small amounts of under-correction (when the correcting effect of the lens nucleus has not been enough) and also actually of over-correction (when the lens nucleus has had too big an effect). In quite a number of cases the amount of spherical aberration is negligible even with pupils of 4 mm. diameter. With larger pupils there is probably a certain amount of under-correction, but this again is less than would be found in the case of spherical surfaces, because the more peripheral parts of the cornea are flattened and therefore refract less (as Gullstrand has shown). We may say, therefore, that in everyday life the effects of spherical aberration are altogether negligible, compared with those of diffraction and chromatic aberration.

Peripheral Aberrations of the Eye. So far the definition of an image lying on the principal axis of the lens has alone been considered. When this is not the case, other conditions are encountered which are more unfavourable in their effects. In the first place, the rays that form images on the peripheral parts of the retina make considerable angles with the surfaces of the eye media. This will cause chromatic difference of magnification, since blue rays will be more bent and will therefore form smaller images than red rays. It will also introduce 'comma,' that is, the effect due to non-compliance with the sine condition. It is seen at once, therefore, that the image formation by the periphery of the eye is altogether more imperfect than it is at the centre. The presence of the nucleus of the lens still further impairs the marginal definition. In fact we may say that in the eye, as in the microscope objective, the marginal images have been sacrificed in order thereby to improve the central ones. That this has been a very valuable result will be shown later. It will be shown in the next section that the most sensitive region of the retina is not exactly in correspondence with the optical axis of the lens system of the eye, being displaced approximately 0·5 mm. to the temporal side.

Structure of Foveal Image may be determined approximately by considering in turn the effects of the different aberrations on the light rays which enter the eye. For this purpose the only errors of importance are chromatic differences of focus and magni-

fication. In addition, however, we must take into account the very important effects of diffraction. The final results of such a calculation show that the images of rays of different wavelength overlap one another. At the centre of the image is seen the sharp yellow focus of the highest intensity. Eccentric to it, and overlapping one another, are seen the diffuse red and green foci, which are of much less intensity. Where these overlap they produce a compound yellow according to the rules of colour mixture. Further outwards is the still more diffuse image of the blue rays, which is of almost negligible intensity. It is seen therefore that the centre of the image is entirely occupied by the sharp and intense focus of the yellow rays. Not only are these rays the brightest in the spectrum, but they are also those nearest to white light in their physiological properties. It is because of this structure of the image that the acuity of vision is so great at the fovea.

Peripheral Images have, as stated above, been to a considerable extent sacrificed, so far as their definition is concerned, in order to obtain the best possible conditions at the fovea. We find, therefore, at the periphery images that in no way compare with those formed near the optical axis of the eye. Even here, however, there is evidence that the eye has been designed to give the best results obtainable.

Scattered Light. In describing the histology of the retina it will be shown how generously the layer of cells lying immediately outside the sensitive layer of rods and cones is supplied with pigment; the object of this is clearly to absorb scattered light. In spite of this, however, we find considerable amounts of light being reflected back again by the retina; in fact it is this light that enables us to see the retinal nerves and vessels through the ophthalmoscope (see p. 427).

ABNORMAL REFRACTION OF THE EYE

It would almost be anticipated that such a complicated organ as the eye would be found to show individual abnormalities. A further consideration would probably suggest to us that, considering the smallness of the change that is necessary in any one of the optical media in order completely to destroy definition, it is nothing short of astonishing that abnormality of refraction is relatively so uncommon. In the newly-born the eye is almost always long-sighted (hypermetropic); this is due to the eyeball being too small for the optical system which it contains; the image formed by the latter is therefore focussed behind the retina. As age advances the eyeball grows until the point is reached at which the eye is emmetropic (normal). If, however, the child is allowed to use its eyes too much for near work, the eyeball goes on increasing in size until it has overshot the mark, and has thus caused the eye to become short-sighted (myopic). There would appear to be some kind of automatic control, which causes the eye to grow till it is in adjustment with the conditions most frequently encountered. This hypothesis is confirmed by the fact that if a child which is beginning to develop short-sight is prevented from using near vision for a year or two, the development of short-sight stops. The importance of the early detection of the onset of short-sight therefore cannot be too strongly urged.

The Methods of Diagnosis. The detection of errors of refraction in the eye may be effected in various ways, each of which is said to possess advantage. Some of these have come into such general use that they may be briefly considered as an introduction to the description of the more important types of error which they are used to investigate.

The Determination of the Visual Acuity. It has been found by experiment that persons with normal sight can distinguish between objects when the angle separating them is not much less than one minute. Test type has therefore been prepared in which the letters are composed of lines which subtend this angle at the eye, when the type is placed at a standard distance of six metres. Persons who are able to read the type at this distance are said to have normal vision. Above these standard letters are placed a series of larger letters, which at two, three or four times the standard distance would subtend

the standard angle. A person with reduced acuity might be able to read at six metres the type that should be read at sixty. He therefore has visual acuity one-tenth the normal. Such a person might have long-sight, short-sight or astigmatism : to determine which is present, there is placed before his eyes a lens-frame, into which can be inserted any two of a large selection of glasses of different power, which are known as trial lenses. These are tried in turn in an orderly manner, until some are found which allow the man to read the standard type at the standard distance. His visual acuity is now at the normal and the strength and shape of the glasses in front of his eyes is carefully noted, so that others of the same power may be fitted to spectacles for him to wear. If the glasses are found to be convex (plus), then he was suffering from long-sight (hypermetropia), and if concave (minus) from short-sight (myopia). Very great care must be taken in this latter case, however, not to prescribe a stronger lens than is necessary. But if, on the other hand, cylindrical lenses had to be used, then he had astigmatism, either alone or in conjunction with long- or short-sight.

THE METHOD OF RETINOSCOPY. Such a method as that just described could only succeed if the person tested were an intelligent adult, because we depend entirely on his giving the correct answer, when we ask if the substitution of a different lens for the one we have already placed before him makes vision better or worse. With a child such a method could never succeed. Another method is therefore practised, which has the great advantage of being independent of the patient; in fact for the purpose of the test he might be blind. This method consists in throwing, into each of his eyes in turn, a beam of light reflected from a plane mirror, in the centre of which is a hole through which the observer looks. When the beam of light is directed into the patient's eye, the observer sees through the pupil a pink reflected beam of light As the mirror is gently tilted, so as to throw the beam slightly upwards and slightly downwards, so the pink beam appears to move up and down behind the patient's pupil. If it moves down as the mirror is tilted down, the movement is said to be WITH the mirror, and the patient is hypermetropic, requiring plus spectacles. If on the other hand the beam moves 'AGAINST the mirror,' minus spectacles are required, since the patient is short-sighted. By placing glasses of different power in front of his eye, until one is found which causes the pink beam to move neither with nor against the mirror, the actual power for the spectacles required by the patient is ascertained. It should be carefully noted however that, since the observer is standing at about a metre distance from the eyes of his patient, plus one D must be subtracted from the power of any glasses that are found to be necessary. Thus if the patient was found to be myopic and minus 7 D spherical lenses were required to neutralise the movement of the beam, then the power that should be ordered is minus 8 D spherical. This test is found to work admirably in practice. It is usual to paralyse the pupil reflex and the accommodation of the patient previous to the test by the use of atropine, but some say that this is unnecessary.

OTHER METHODS. Of other methods of testing vision little need be said: some require the use of special instruments such as the optometer and the refractometer. Another again depends on the determination of both the far and near points. This is of distinct value because it at once gives the amplitude of the accommodation, which is an important determination. Others are based on the use of the ophthalmoscope. But none of these methods is so simple or accurate as the method of retinoscopy described above.

STENOPÆIC APERTURE. Often in practice the question arises as to whether low visual acuity is due to defect in the optical media of the eye, or to disease of the retina. This question can be readily answered by placing in front of each eye in turn a metal disc in which has been drilled a hole 0·3 mm. in diameter. If this improves acuity the defect is not in the retina ; if it does not, it is. This test should be done in a good light because of the small amount of light passed by the hole. A hole used in this way is called a stenopæic aperture.

HYPERMETROPIA OR LONG-SIGHT. There are two principal varieties of long-sight: firstly, that in which the eyeball is too small and too short for the normal optical system, secondly, that in which the eyeball is normal but the refracting power of the lens below the normal. The first variety is found in childhood, because the optical system reaches its adult size much earlier than does the eyeball. In the majority of children the eyeball continues to grow until it is the correct size, and therefore long-sight disappears. In a certain number of cases this does not happen, and therefore long-sight remains through life. The second variety is found in old age, and appears to be due to the absorption of water by the lens. The result in both cases is that the rays of light from distant objects are brought to a focus behind the retina, and therefore in order to focus them the accommodation has to be used (see Fig. 257). It follows from this that there is

less accommodation remaining for the focussing of near objects, and therefore an inability to see distinctly at relatively short distances from the eye. Thus the use of the term long-sight.

Hypermetropia in adults is, therefore, more an abnormality than a disease; it causes a disposition, however, to three more serious conditions, namely glaucoma, internal strabismus and eye-strain. Glaucoma has already been described (see p. 399); it is due to an abnormal rise in the intraocular pressure, which occurs owing to the free escape of the aqueous humour at the filtration angle being checked. Now in hypermetropia we have seen that the eyeball is too small for its optical apparatus, and therefore the lens occupies too much of the space in the small anterior and posterior chambers. This causes the ciliary bodies and roots of the iris to be squeezed and greatly reduces the space at the filtration angle. An attack of glaucoma is therefore more liable to occur in the hypermetrope than in a person with normal refraction.

Internal strabismus is caused in hypermetropia by the accommodative effort that is made in order to focus an image on the retina because, as we have seen above (p. 391), convergence and accommodation are associated actions. When, therefore, the long-sight has been corrected by means of spectacles, and the accommodation is no longer called into play for seeing at a distance, the associated convergence no longer occurs and the strabismus disappears.

Eye-strain is caused in hypermetropia by the continual call for accommodation.

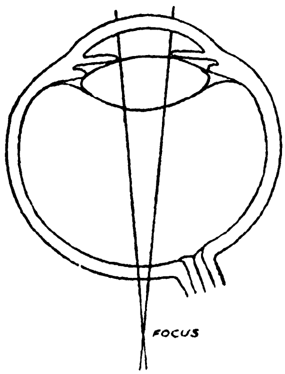

FIG. 257. Hypermetropic Eye.
The eyeball is too short, and therefore rays from a distant object come to a focus beyond the retina.

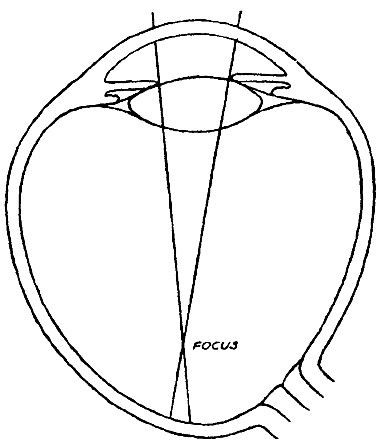

FIG. 258. Myopic Eye.
The eyeball is too long, and therefore rays from a distant object come to a focus in front of the retina.

Further, this must occur without convergence, for otherwise diplopia (seeing double) and strabismus develop as just described. A special strain is therefore placed not only on the ciliary muscles, but also on the external eye muscles. This state of affairs very rapidly causes fatigue, and headaches are therefore common.

The treatment of long-sight consists in prescribing suitable convex spectacles. It should be noted that the amount of long-sight actually present is shown only when the accommodation has been paralysed by atropine, because the patient has grown so accustomed to use his accommodation in ordinary vision that he is unable voluntarily to relax it. There is a certain amount of spasm of the accommodation. Because of this, the glasses prescribed should be less strong at first than the full correction shown to be necessary. These may be substituted by more powerful ones later.

MYOPIA OR SHORT-SIGHT. In this condition parallel rays, that is those coming from distant objects, come to a focus so far in front of the retina that the image appears blurred (see Fig. 258). Myopia may be caused in two ways, which are similar but opposite in action to those that cause hypermetropia; the first type is caused by the eyeball being too long, and the second by the refraction of the lens being too high. The former, which is the more common, usually develops in youth, particularly at the school age when the growth of the body makes special demands on the system, and at the same time feeding is usually bad. The constant use of the eyes for near work

causes them considerable strain, which they are unable to withstand owing to their being unable to compete for nourishment with the rest of the body. The choroid and sclera therefore become thin, are no longer able to stand the tension set up by the intra-ocular pressure, and therefore expand, causing the eyeball to become larger than normal, and taking the retina beyond the focus of the optical system (Fig. 259). The treatment of myopia is therefore not only the wearing of spectacles, but the absolute prohibition of near work or close study, the administration of extra-nourishing food and an open-air life for a year or more. If these steps are taken at once, the myopia may get no worse, and may in fact get better. But if neglected the condition will almost certainly get worse. As myopia is a disease particularly liable to occur at the school age, schoolmasters and others associating with children should be on the look out for conditions likely to cause it, such as bad light, bad food and poor ventilation, and for its presence in any of the children. Glasses should always be prescribed, and care taken that the child wears them constantly, because it is found that beside assisting good definition and relieving eye-strain, they actually tend to check the further development of the trouble.

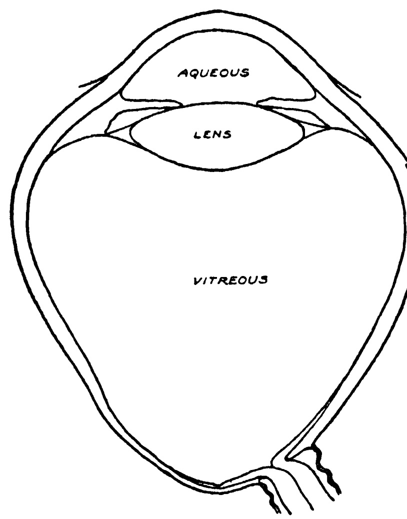

Fig. 259. The Asymmetry of the Eyeball and Kinking of the Optic Nerve caused by High Myopia.

Certain complications sometimes attend myopia; these are divergent strabismus, eye-strain, and spasm of the accommodation.

The divergent strabismus has a similar origin to the convergent strabismus met with in hypermetropia, namely association of deviation of the eye axes with the adjustment of the accommodation. Now since, in the normal individual, the relaxation of the accommodation of the eye is associated with parallel axes of the eyes (in order to look at distant objects), in myopia the disuse of the accommodation for near vision causes the eye axes to remain straight and therefore produces the effects of an external strabismus. The use of glasses introduces again the necessity of accommodation, exactly as if the eye were normal, and therefore abolishes the strabismus. In the majority of cases an actual strabismus does not develop, but there is nevertheless a strong tendency to diplopia, especially when the eyes are tired. The eye-strain which frequently accompanies myopia probably has its origin in the effort to converge the eye axes without at the same time calling the accommodation into play.

Spasm of accommodation frequently accompanies myopia, and has the effect of making the myopia seem greater than it actually is. The true state of affairs is at once found when atropine is used, because the accommodation is thus abolished. Sometimes in children spasm of accommodation occurs without any actual abnormality of refraction. Such cases should be treated with the same care as those that are already developing myopia.

Astigmatism. The condition of the eye called astigmatism is one in which parallel rays are not brought to a focus in a single plane, but in a number of different planes. There are two different varieties of astigmatism. In the first, or irregular, variety the separate parts of one meridian of the eye form different foci. This is found to occur during the development of cataract in the crystalline lens, and also after ulceration of the cornea. The effects of this form of astigmatism on vision vary with the severity of the condition; in moderate cases a frequent phenomenon is the formation of a double image in the affected eye. Glasses as a rule do not give benefit. In severe types the use of a stenopæic aperture may improve definition.

In the second variety, or regular astigmatism, the parts of any one meridian give the same focus, but the different meridians have different foci (Fig. 260). There are, however, two meridians at right angles to one another, one of which has the longest and the other the shortest focus, the meridians in between showing an orderly sequence between these two extreme values. Thus the use of the term "regular astigmatism." Two types of patient are found to suffer from this condition, those who have inherited and those

who have acquired it as a sequence to injury, operation or disease. The effects on vision are varied, but the characteristic features are distortion of objects looked at, and

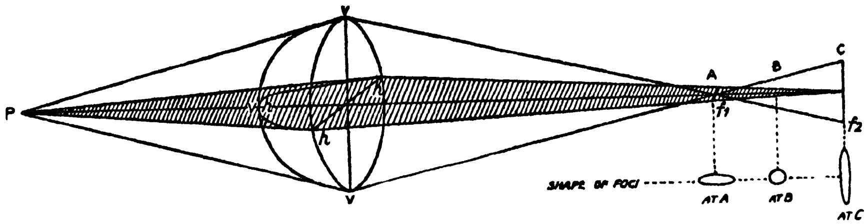

FIG. 260. Showing the Shape of Foci at different Positions in cases of Astigmatism.

indistinctness of lines in one direction, while those at right angles are quite sharp. Headaches, eye-strain and dimness of vision are very common. Many types are met with because the maximum and minimum meridians may occupy any angle, so long as they are at right angles to one another, and there may be any accompanying degree of myopia or hypermetropia.

The diagnosis and measurement of astigmatism present no difficulties. Its existence may be readily proved by causing the patient to look at a figure consisting of a series of lines radiating from a common centre. It is then found that while some of the lines are sharp those at right angles are indistinct. This test also shows the axes of the principal meridians. By retinoscopy (see p. 414) the axes and the amounts of the abnormality in those axes may be readily determined. The treatment consists in giving spectacles which have been ground on one side to a cylindrical surface. The axis of this cylinder is adjusted to correspond with one of the principal meridians of the eye of the patient. The curve given to the cylinder is that which will cause the focus of the meridian with which it corresponds to be equal to the focus of the other meridian. The other side of the spectacle lens is ground to that spherical surface which will make the eye emmetropic after it has been corrected by the cylinder.

ANISOMETROPIA. The last abnormality of refraction which we have to consider is called anisometropia. It simply means difference between the refraction of the two eyes. The effect on vision is very slight, since it is found that as a rule one eye does all the work, and the image of the other, which is necessarily indistinct, is prevented from reaching consciousness. The result in course of time is that the unused eye loses to a considerable extent its power of seeing and as a result strabismus develops. Treatment consists in giving glasses which correct each eye separately, and then instituting exercises for the poorer eye, in order to improve its vision. The results of this treatment are good.

6. THE RETINA

The retina is a delicate membrane lying inside the choroid coat of the eye. Its internal surface lies in contact with the hyaloid membrane of the vitreous body. It is thus supported on both sides. The retina itself consists of two layers, the outer or pigmented, and the inner or nervous. Whereas embryologically the retina covers the whole internal surface of the eye including the ciliary processes and the iris, this is not the case with the nervous layer, because this stops near the equator of the eye at the ora serrata, and is here replaced by a layer of columnar epithelium. Opposite the pupil a yellow spot is seen on the retina, the *macula lutea*, and in the centre of this there is an oval depression, the *fovea centralis*. The optic nerve enters the eyeball through an aperture in the sclera and choroid, and then passes through the posterior surface of the retina to spread out over the internal surface. In the fovea, however, this is not the case, for the depression at this point is caused by the absence of nerve fibres. The point at which the optic nerve enters the eye is easily recognised from inside the eyeball because the numerous white nerve fibres, as they bend over the edge of the aperture in the retina, form a characteristic white mound called the colliculus, at the centre of which is a depressed portion called the optic cup. It is in the centre of this cup that the central artery of the retina and the corresponding vein first make their appearance. These have the important function of nourishing the retina; the additional blood supply through the intimate contact between the retina and the vascular choroid, although important, is quite insufficient to supply the needs of vision, as is shown by the immediate and permanent blindness which follows blocking of the central artery of the retina.

When sections of the retina are examined under the microscope it is found that they consist of the following layers from without inwards (Figs. 261 & 262) :—

1. Layer of pigmented epithelium	Developed from posterior layer.
2. Layer of rods and cones (bacillary layer) 3. Outer nuclear or granular layer 4. Outer molecular or plexiform layer 5. Inner nuclear or granular layer 6. Inner molecular or plexiform layer 7. Layer of ganglion nerve cells 8. Layer of nerve fibres and vessels	Developed from anterior layer of optic vesicle.

In order to understand the structure of these layers, it is necessary to keep in mind the fact that the optic nerve and cup are outgrowths of part of the brain. We

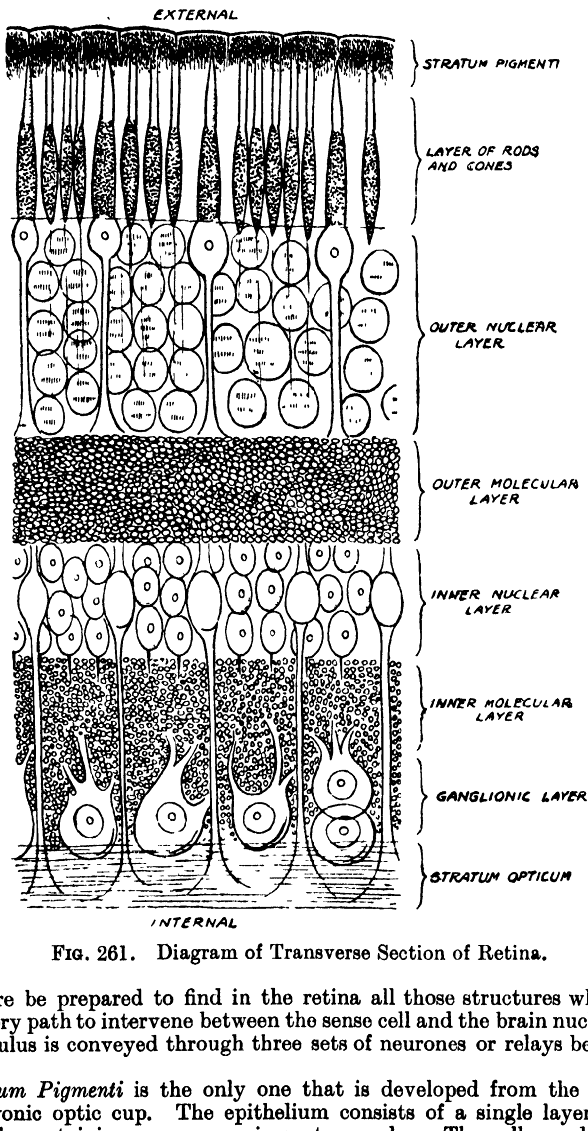

FIG. 261. Diagram of Transverse Section of Retina.

must therefore be prepared to find in the retina all those structures which are found in every sensory path to intervene between the sense cell and the brain nucleus. In every case the stimulus is conveyed through three sets of neurones or relays before it reaches the brain.

The Stratum Pigmenti is the only one that is developed from the external layer of the embryonic optic cup. The epithelium consists of a single layer of hexagonal nucleated cells containing numerous pigment granules. The cells send fine processes

between the limbs of the rods. The bases of these cells are firmly attached to the choroid, and thus give support to the rest of the retina.

The object of these cell processes and the pigment granules which they contain would appear to be either the prevention of an image formed on one part of the retina from spreading to the sensitive elements of surrounding portions, or else the protection of these elements from excessive light action. But it has been definitely proved that the cells themselves have another and important function to perform, namely the secretion of the pigment called visual purple (rhodopsin). The important functions of this pigment will be described later.

The Bacillary Layer of rods and cones is separated from the succeeding layer by the external limiting membrane. Each rod and cone consists of an outer and inner limb, the forms of which are well shown in Fig. 263. It will be seen that the outer limbs are striated, the cones coarsely, the rods finely; they tend after hardening to break up into discs. The inner limbs of both rods and cones have a strong affinity for dyes.

The Outer Nuclear Layer consists of the cells of the first order neurones or the granules

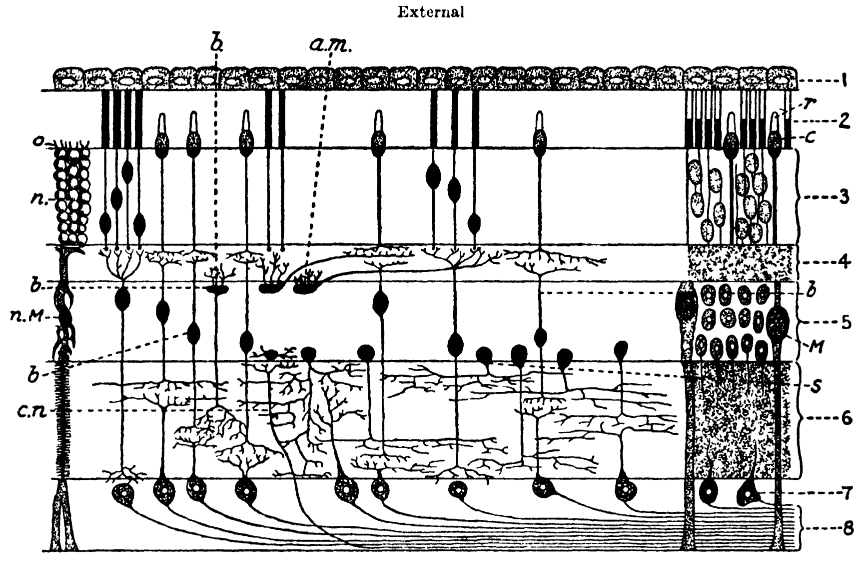

FIG. 262. Diagram to show the different Cellular Structures found in the Retina.

of the rods and cones. The cells are nucleated, somewhat smaller than the bipolar cells, and their nuclei are striated. They give off two processes, one of which forms dendrites in the fourth layer, the other connects with either a rod or cone as the case may be (see later).

The Outer Molecular Layer is much like the inner; it consists of the dendrites of the second order neurones and the first.

The Inner Nuclear Layer largely consists of bipolar second order neurone cells. There are, however, also present the nuclei of the horizontal cells, and also the nuclei of similar cells (amacrine cells, *a.m.*, Fig. 262), whose dendrites travel in the outer molecular layer. The bipolar cells, which are fusiform in shape and nucleated, are of three kinds: (*a*) those which connect with rods, (*b*) those which connect with cones, and (*c*) giant bipolars which connect with either.

The Inner Molecular Layer consists of a feltwork made up by the interlacing dendrites of the ganglion cells with those of the inner nuclear cells or second order neurones. There are also the dendrites of horizontal cells or spongioblasts (*s*, Fig. 262). These possibly serve to associate the impulses from different parts of the retina, such as is supposed to occur in the brain. It should be noted that they appear to be absent from the fovea and macula.

The Ganglion Cell Layer consists of a single layer of large oval cells. These are nucleated and give off the axons which we have already described, and a bunch of

dendrites which ramify with others in the inner molecular layer. Only at the macula is more than one layer of ganglion cells present; this is due to their almost complete absence at the fovea. The macula therefore not only has its own relays but those of the fovea as well.

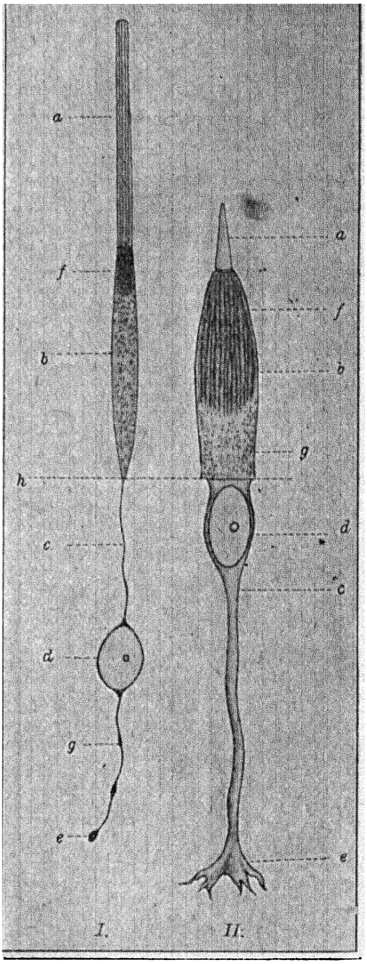

FIG. 263. (R. GREEFF.)
I. A Rod.
II. A Cone of Mammalian Retina.
h. External Limiting Membrane.

The Nerve Fibre Layer (stratum opticum) consists of the non-medullated axons of the large ganglion cells found in the second layer. These axons are the third order neurones which become myelinated after they have passed out of the eyeball, and travel by paths to the calcarine cortex described on p. 319 Beside the fibres conveying visual impressions there are others which belong to the pupillomotor reflex. Others again bring impulses from the brain to the retina; their functions will be considered below.

It should be noted that beside the structures described above, which have the functional activities of the retina to perform, there are a number of connective tissue elements which form the retina into one coherent structure. Since the retina is developed from an outgrowth of the brain, these structures are similar in type to those met there; we therefore find neuroglia and also long cells which extend through the first seven layers and hold them together, namely the fibres of Muller (*M.* and *n.M.*, Fig. 262).

THE DIFFERENT PARTS OF THE RETINA show marked variation in detail. At the fovea (Fig 264) cones alone are found; only one of these connects to each axon. The structural peculiarities of the fovea may be summarised as follows:—(1) Absence of rods. (2) The cones are longer, more highly developed, and some say more rod-like than those found elsewhere. They are very closely packed, so that their inner limbs are seen in transverse section to have a hexagonal shape, the flat surfaces being in contact with those of their neighbours. (3) The rows of nerve cells and dendrites, which in the rest of the retina are approximately in line with the rod or cone to which they belong, are in the fovea pressed to one side in a direction away from the centre. In this way a cone may have the nerve cells to which it is connected placed at a considerable distance away in the surrounding macula. It is this displacement of the nerve fibres and their cells that causes the fovea to appear hollow. The purpose of this physiological arrangement would without question appear to be the avoidance, at this important region of the retina, of the scattering of the image which passage through the nerve cell layers would introduce. (4) The fovea, unlike the rest of the retina, is devoid of blood vessels. The purpose of this arrangement would appear to be similar to that just described. (5) Visual purple is said to be absent from the fovea. This would appear to be connected with the absence of rods.

Round the fovea is a ring in which rods and cones are present in almost equal number. In still more peripheral regions cones are relatively few, and several rods connect with each axon; this reduces the relative number of nerve fibres.

CHANGES IN THE RETINA ON EXPOSURE TO LIGHT

A light stimulus falling on the retina causes a number of changes to occur which may be classed as structural, physical, chemical and physiological.

STRUCTURAL CHANGES occur on exposure of the eye to light: firstly, movement of the pigment from the outer epithelial layer into the spaces between the rods and cones; secondly, shortening of the cones themselves (Fig. 265)

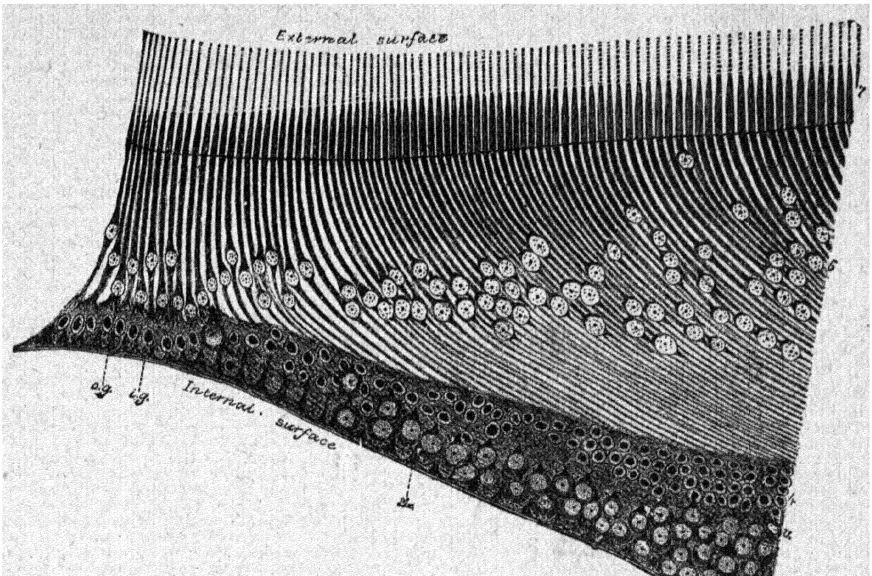

FIG. 264. Section through half the Fovea Centralis. (SCHAFER and GOLDING BIRD.)

These changes occur only when the connections of the eye with the brain are intact. The rate of movement appears to vary with intensity, and violet light is said to be more effective than red. It is interesting to find that

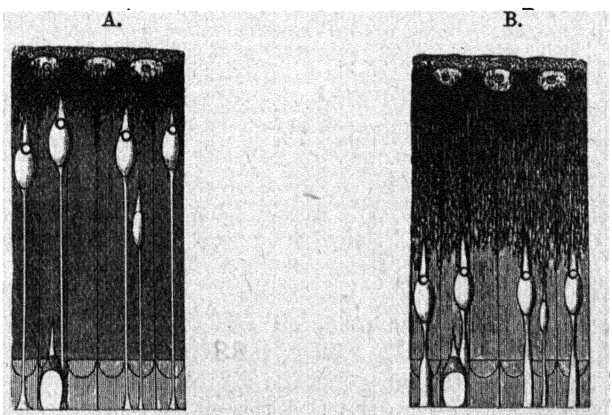

FIG. 265. Sections of the Frog's Retina.

A. Kept in the dark. B. After exposure to the light, showing retraction of the cones, and protrusion of the pigmented epithelium between the outer limbs of the rods. (ENGELMANN.)

electrical stimulation of the optic nerve or the falling of light on the other retina to that of the eye observed also causes these cone movements. It is supposed that the impulses which effect these movements travel through the nerve fibres already described as descending from the brain to the retinæ; it is for this reason that Engelmann called these fibres 'retinomotor.'

Other structural changes that are found, by histological investigation, to follow exposure of the retina to light, are swelling of the outer limbs of the rods, and the disappearance of chromatin granules from the ganglion cells. Both these changes are said to occur more rapidly under the action of rays of short wavelength.

PHYSICAL CHANGES are also found when the retina is stimulated by light, namely an electrical response somewhat similar to the current of action in nerve. A typical curve is shown in Fig. 266. A study of the electric response to lights of different colours and intensities has given the following results. With light of any one colour, a geometric rise of intensity causes an arithmetic increase in the current. With coloured rays of apparent equal intensity, yellow rays are said to give a larger current in the light adapted eye, and green in the dark adapted eye. It is interesting to observe that the current commences after a latent period, which is of the same order as that found for the perception of light by the eye. This and other facts mentioned above, would seem to point to the currents observed being the accompaniment of the passage of the nervous impulses to the brain.

CHEMICAL CHANGES in the retina on exposure to light are of two kinds: firstly a tendency of the retina as a whole to become acid in reaction, as

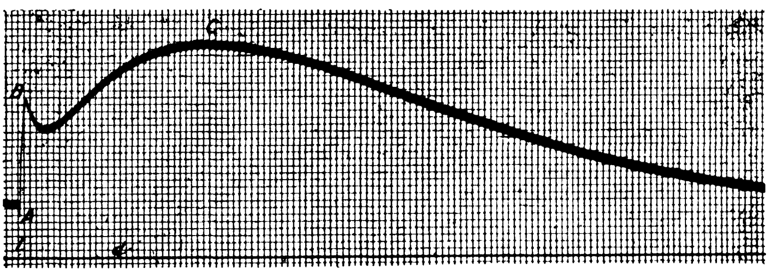

FIG. 266. Electrical Variation in Frog's Eye as recorded by the String Galvanometer. (EINTHOVEN and JOLLY.) On exposure to a single flash.

shown in the change in its behaviour to certain stains; and secondly the bleaching of two pigments, namely the *visual purple* and *fuscin*. With regard to visual purple (or rhodopsin) a large number of facts have been made out. In the first place it is found in association with the rod retinal structures only, and is therefore absent from the human fovea centralis. It is a derivative of vitamin A, and when bleached by light liberates various products related to the carotenes.* Transient orange first appears and then indicator yellow (Lythgoe).† Bleaching on exposure to light occurs in solution as well as in the retina. According to Wald exposure to light causes visual purple to split into a yellow pigment 'retinene' and a protein. Retinene on further exposure to light yields a colourless substance—visual white. When vitamin A is present in the retina the protein can combine with it to form visual purple.

The absorption of light by visual purple is greatest for the middle of the spectrum; the transmitted colour is therefore composed principally of red and violet rays.‡ Small variations of colour are found, however, in samples obtained from different animals; some are even rose-pink in colour. In the second place the most potent colours in producing bleaching are those near

* WALD. *J. Gen. Physiol.*, 1935, **19**, 351.
† LYTHGOE & QUILLIAM. *J. Physiol.*, 1938, **94**, 399.
‡ LYTHGOE. *J. Physiol.*, 1937, **89**, 331.

the centre of the spectrum, that is the rays which are most strongly absorbed. This pigment therefore obeys Draper's law, which states that those rays that are absorbed are those which produce chemical change.

It will be shown below that the retina when adapted for vision in dim light is not only colour blind, but also that the green rays of the spectrum appear to have the maximum luminosity. It will also be shown that the rod-containing parts of the retina, where visual purple is to be found, are the only regions that react to light of low intensity. Moreover, the curve which represents the rate of bleaching of visual purple by rays of different colour is very similar in shape to that which shows the visibility of lights of low intensity, as in Fig. 267. The obvious inference is that visual purple is concerned with twilight vision. Accordingly it is found that animals deprived of vitamin A, a precursor of visual purple, show deficient twilight vision (night-blindness).* When the visual purple in the retina has been bleached by exposure to light, there follows a gradual re-formation of purple, which is independent of nerve connections, but occurs only so long as the stratum pigmenti is in contact with the rod epithelial layer. If we suppose that the product formed by the bleaching of the visual purple stimulates the rod apparatus, causing it to send impulses to the brain, we have a plausible hypothesis of the mechanism for night vision. This we may briefly describe as follows :—

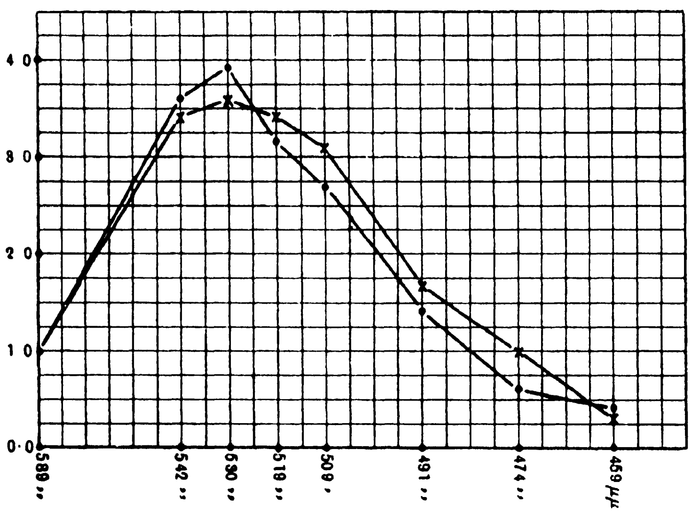

FIG. 267. Shows the Similarity between the Curve representing the Rate of Bleaching of Visual Purple by Light of different Wavelengths and the Luminosity Curve of Twilight Vision.

When light falls on the retina, certain rays, particularly those near the middle of the spectrum, are absorbed by the visual purple. The pigment is bleached in proportion to the light absorbed, forming a new product : this acts on the rods, causing them to send impulses to the brain which continue so long as the light falls. When the light stops the stimulating product disappears again and therefore the stimulus to the rods ceases.

The work of Hecht (1920–21) is very strongly in favour of such a view. He has shown that the rate of bleaching of a solution of visual purple by light and the rate of light adaptation of the human eye both follow a logarithmic law : that is, the 'reaction'

* TANSLEY. *J. Physiol*, 1931, 71, 442.

in both cases appears to be one involving the splitting up of a single large molecule into components. On the other hand, the rate of dark adaptation of the human eye follows a curve similar in shape to that representing the rate of regeneration of visual purple.* Taken in conjunction these pieces of evidence point most strongly to the conclusion that a single large molecule of visual purple splits under the action of light into components, and that these when the light is removed combine again to re-form visual purple. Many analogous photochemical systems have been studied.

A picture, formed by the bleaching of the visual purple in those parts of the retina which correspond to the high lights of the image formed by the lens, can be fixed, much like a photograph, by immersing the retina in a solution of alum.

Fuscin is the pigment found in the form of needles, plates or prisms in the processes of the cells of the stratum pigmenti of the retina. The object of this pigment is apparently to absorb light, which might tend to spread from those retinal elements on which an image of a light source is falling to neighbouring ones. Some of this pigment is bleached by strong light, but so far as is known the break-down products have no visual function. The presence of other pigments has been described in the retina, such as visual yellow and the bright pigment granules found in birds. The presence of the latter is too variable for them to be considered to take any essential part in the visual mechanism.

Physiological changes produced by light depend greatly on the region of the retina on which it falls, since this may contain rods only, cones only, both rods and cones, or neither rods nor cones. The peripheral parts of the retina contain numerous rods and very few cones. When stimulated by light of low intensity, this part of the retina is found to be exceedingly sensitive, particularly if the eyes have been closed or kept in the dark for a time. Tests with light of low intensity and of different colours show that the region is colour blind, but that rays in the middle of the spectrum are more readily appreciated than others. We have here well developed the so-called 'twilight vision,' which is associated with the rod-visual purple mechanism just described. Besides being very sensitive to light of low intensity, the periphery of the eye is also particularly perceptive of light of short duration. This part of the retina, therefore, appreciates movement at night very readily. Lastly, owing to the fact that a number of rods connect with one nerve fibre conveying the impulses to the brain, the periphery of the retina has a poor perception of detail.

The Fovea Centralis is found to contain cones only. The vision in this region is therefore the antithesis of that found at the periphery. The appreciation of light of low intensity is bad, but when an image is sufficiently bright to cause stimulation, its colour is perceived. When light is poor, rapid motion is not so well observed as it is by the periphery. There is an extraordinary acuteness in perceiving fine detail. This is due to the fact that the cones in the fovea are very closely packed, so closely that they become flattened where they touch one another and thus have a hexagonal shape in transverse section. Further, each cone is connected to its own nerve fibre, so that no cyphering of the impulses can occur on the way to the brain. Experiments on visual acuity definitely show that the fineness of the detail, which the eye can perceive at the foveal region, is fully as great as that which we should expect to find, if each cone acted quite independently of its neighbours. Parts of the retina around the macula lutea, since they contain both rods and cones, possess, as we should expect, both the power to perceive colour found at the fovea and the ability to react to light of low intensity,

* Tansley. *J. Physiol.*, 1931, **71**, 442; *Proc. Roy. Soc.*, 1933, **114B**, 79.

without colour vision, which is possessed by the periphery of the retina. The presence of rods scattered between the cones, however, naturally impairs to some extent the appreciation of fine detail. Rays of light which reach the fovea centralis by passing through the centre of the pupil were found by Styles and Crawford to be more effective than rays which have passed through the periphery of the pupil. This phenomenon has been called by them the directional sensitivity of the retina. They suppose it to be due to the narrow cylindrical shape of the foveal cones (see p. 420).

At the papilla where the optic nerve enters the eye there are neither rods nor cones, and therefore, as we should expect, this region is quite blind. This fact can be readily proved by looking with the right eye at the

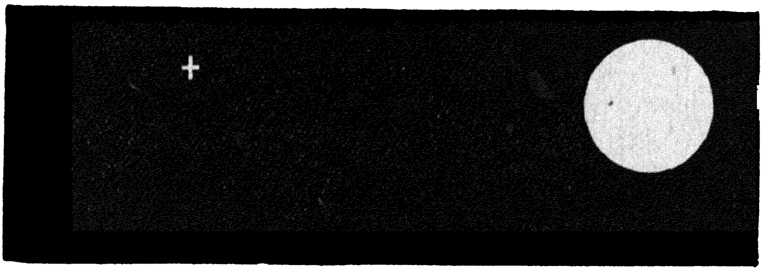

FIG. 268. Look at Cross with Right Eye, hold Book at about 10 inches.

cross in Fig. 268. If now the book be held about 10 inches from the eye the white disc will be found to disappear. By a simple calculation, it is found that the disc corresponds with the papilla of the optic nerve.

THE VISUAL FIELDS. Since the appearance of an external object will vary to a considerable extent, according to the region of the retina on which its image falls, it is a matter of considerable interest to determine the positions at which the appearances undergo change. This is also of practical value because the positions are found to be affected by disease. The determinations are usually made by means of an instrument called a *perimeter*.

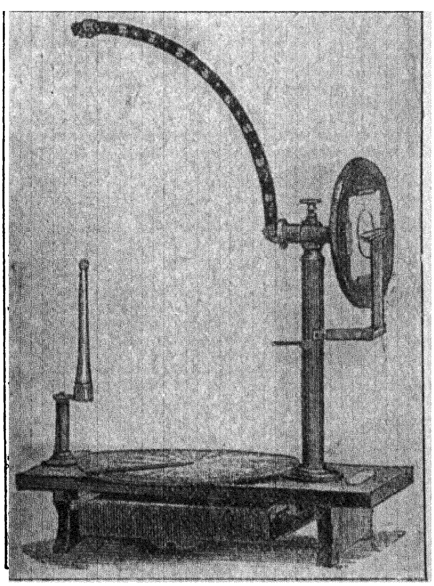

FIG. 269. Priestly Smith's Perimeter.

This consists (Fig. 269) of a metal arm bent to the segment of a circle. This is so mounted in relationship to a horizontal bearing that the segment always has its centre in correspondence with a fixed pointer which is seen on the left of the diagram. If the eye of the patient is applied close to this pointer and looks towards the centre of the bearing, the degrees marked on the metal segment show the actual angle at which an index is situated in relationship to the eye axis, no matter in what meridian the metal segment may lie. The index mark usually consists of a disc 2 mm. in diameter, either of white or of coloured paper, according to whether the rod or cone area is to be examined.

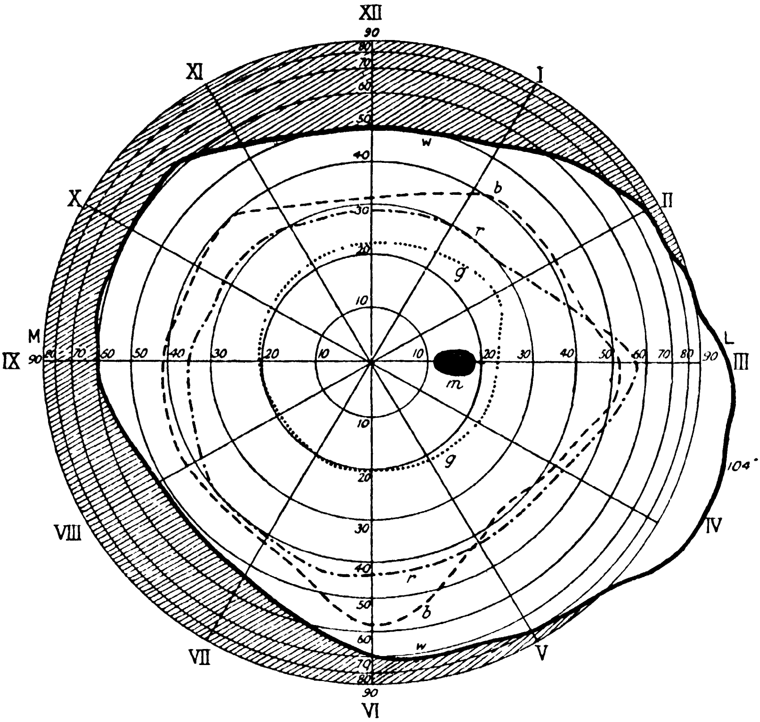

FIG. 270. Field of Vision for White and Colours of a normal Right Eye as obtained by the Perimeter. (HARTRIDGE.)

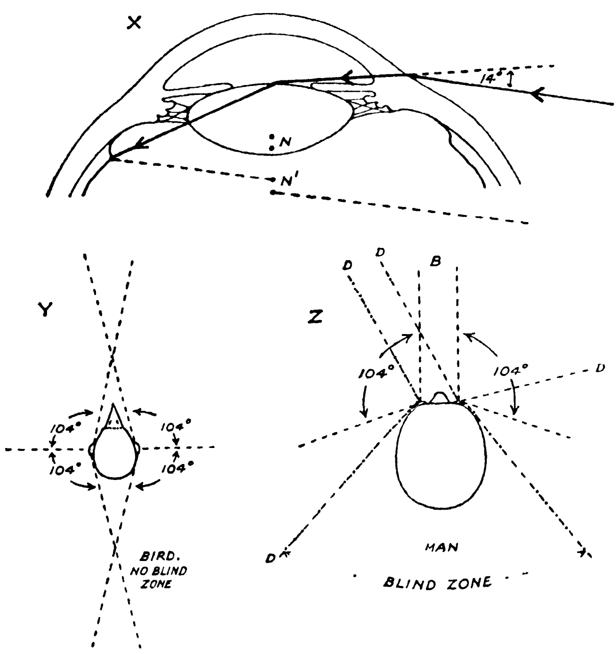

FIG. 271. Diagram Z shows Size of Blind Zone in Man.
Diagram X shows how Rays from the extreme Periphery enter the Eye and reach the Retina.
Diagram Y shows Absence of Blind Zone in Birds and certain Animals. (HARTRIDGE.)

The results obtained by means of this instrument are shown in Fig. 270, which gives a typical curve for the right eye. The shaded area on the diagram is due to the obstruction of the eyebrow, nose and cheek of the patient. The visual field on the outer side will be seen to extend actually 14 degrees beyond the right angle. This result, which at first sight appears to be impossible, is in fact due to the considerable refraction of the light rays that occurs at the extreme edge of the cornea. The direction of the beam of light entering the eye under these circumstances is shown in Fig. 271, X.

It will be observed that, even when looking straight in front, a man can see to a considerable angle behind himself. By deviating the eyes only slightly to either side, this angle can be increased to 40 degrees in the average case, as shown at Fig. 271, Z. This ability to see to a considerable extent behind him is due to the narrowness of the head between the frontal processes and zygomatic bones. In those animals in which the eyes are placed on the sides of the head, and the visual axes are diametrically opposite to one another, the visual fields will actually overlap a short distance from the head, so that there will be no direction from which an enemy can attack without being observed (Fig. 271, Y).

UTILITY OF PERIPHERAL VISION. The high visual acuity of the fovea, and the great facility with which the eyes can be directed so that images form on this region, might raise the question as to the utility of peripheral vision. This question may be investigated experimentally by placing restricting screens in front of the eyes, or by ascertaining the experience of persons who are suffering from blindness in the periphery as the result of disease, *e.g.* retinitis pigmentosa. Both methods show that the periphery is of great value in directing attention to outlying obstacles. Our attention being excited, we direct the gaze in the direction indicated, in order to bring into action the greater power of analysis of the fovea.

CENTRAL CONNECTIONS OF THE RETINA. The nerve paths by which the retina, itself a part of the central nervous system, connects with other parts of the central nervous system has been considered in detail (see p. 303). Briefly the optic nerves from the two retinæ converge and join at the chiasma. These fibres from the nasal halves of both retinæ cross, so that the optic tracts which convey the impulses from the chiasma to their destinations contain fibres which have come from the same side of the retinæ as that to which they themselves belong. The right-hand tract has fibres from the right-hand halves of both retinæ. The fibres pass chiefly to the calcarine region of the cerebral cortex *viâ* the lowest part of the optic thalamus and the most posterior part of the corona radiata for the sense of sight. But some pass *viâ* the superior corpora quadrigemina to the nuclei of the oculomotor nerves, thus causing reflex eye movements.

THE OPHTHALMOSCOPE. If the retina of a patient be illuminated by causing a beam of light to enter the pupil, the reflected light will cause the interior surface of the retina to be visible. In order to see the image distinctly, it is necessary either that both the eye of the patient and also that of the observer should be focussed for infinity (the direct method) or that both eyes should be focussed for one and the same intermediate plane (the indirect method). Both methods should be practised, since the indirect method gives a low magnification while the direct method gives a high one. The indirect method is carried out as follows:—A bright source of light having been placed behind and slightly to one side of the patient, the observer standing about half a metre in front of him reflects by means of a concave mirror an image of the light into his pupil. At the centre of the mirror is an aperture through which he sees the light which is reflected back from the patient's retina. The observer now holds a biconvex

lens of about 6 cm. focal length about 8 cm. in front of the patient's eye, while he still directs the beam of light into the pupil as before. The image of the retina, which would normally be focussed by the lens system of the patient's eye at infinity, is now brought

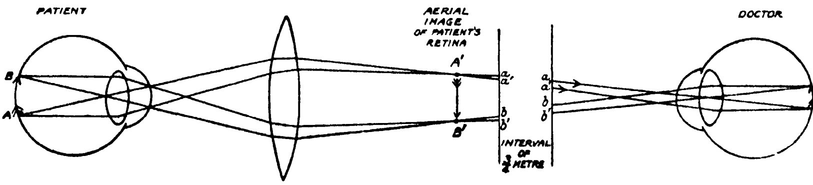

FIG. 272. Diagram to show Paths of Rays from Eye of Patient to Observer when the Indirect Method of Ophthalmoscopy is in use. (HARTRIDGE.)

to a focus by the convex lens, forming what is called an aerial image (see Fig. 272). It is this image that the observer sees (Fig. 273), and in it are shown all the particular features of the vessels and nerves of the patient's retina. Beside its very great utility to the oculist, the ophthalmoscope is a very valuable instrument to the physician, for retinal vessels and nerves frequently show the evidences of constitutional disease, which are of great assistance to diagnosis. Ophthalmoscopes are usually fitted with a number of small lenses of graduated power, which may be introduced as required behind the mirror. For the indirect method they are seldom required. The magnification of the retina given by the indirect method is usually about 3, while that provided by the direct is 12. If a higher magnification is an advantage it may be obtained by the above method by using a biconvex lens of longer focal length (say 12 cm.).

The image seen by the direct method is erect, that by the indirect is inverted.

On examining the back of the eyeball by either of these methods, the most prominent object is the optic disc or optic nerve papilla, which marks the point of entrance of the optic nerve. It is seen as a pale oval disc surrounded by a deep red background (Fig. 273). From the middle of the papilla the retinal vessels pass into the eyeball, and they are seen diverging from the papilla to ramify over the rest of the retina. The arteries can be distinguished from the veins by their brighter red colour as well as by the stronger reflection of light from their surfaces. The yellow spot is very difficult to see, except in atropinised eyes, since it comes into view only when the observed eye is looking straight into the ophthalmoscope. Under these conditions there is a strong 'light reflex,' and the pupil contracts up to a pin-point, unless paralysed by means of atropine.

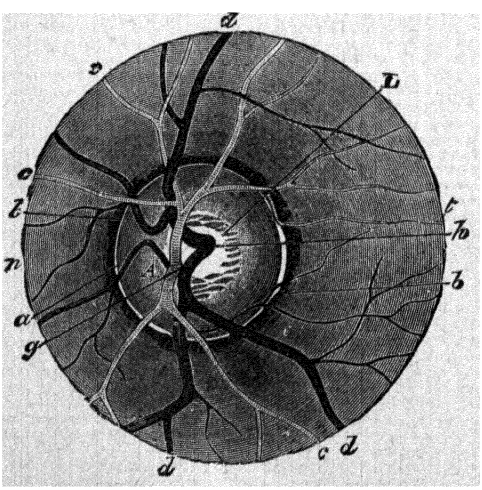

FIG. 273. Ophthalmoscopic View of Fundus of Eye, showing the Optic Disc, or point of entry of the Optic Nerve, with the Retinal Vessels branching from its centre.

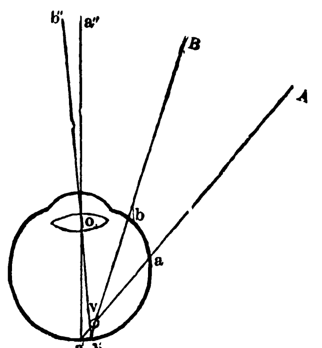

FIG. 274. Diagram of the Path of the Rays of Light in the Formation of Purkinje's Figures.

v represents a retinal vessel. When this is illuminated from A, a shadow is formed on the hinder layers of the retina at a′. This is projected along a line passing through the optic axis, and appears to come from a point (a″) on the wall. On moving the light from A to B, the image of the vessel appears to move from a″ to b″.

In order to see the blind spot, or optic disc, the observed eye must be directed inwards; thus if A is looking at the right eye of B, B must be told to look over A's right shoulder.

By projecting a highly concentrated beam of light on to the side of the eyeball, it is possible to cause sufficient light to pass through the wall for the retina to perceive the stimulus. When that is the case, it is found that the retinal arteries and veins are seen by the subject as dark images on a bright ground (Purkinje's figures). By moving the point of illumination and then measuring the apparent shift of the vessels which occurs, it has been found possible to estimate the depth below the vessels at which the receptive surface of the retina is placed, namely 0·17 to 0·36 mm. Now the average distance between the vessels and the layer of rods and cones is found to be 0·2 to 0·3 mm.; it must therefore be the layer of rods and cones which forms the sensitive layer. The directions taken by the light rays are shown in Fig. 274.

Another method of viewing the vessels in one's own eye is to look through a small hole in a metal plate at a smooth white surface. On oscillating the aperture in relationship with the pupil about once a second, the vessels will be seen as shadows on the bright background.

7. THE RELATIONSHIP BETWEEN STIMULUS AND SENSATION

Since we are unable to express our sensations in terms of physical units (we cannot say, for example, when one source is twice as bright as another), two methods of investigation are alone available, namely that which involves the determination of threshold values, and that which depends on the making of comparisons.

In order that a source of light shall be perceived, the image which is formed on the retina must have certain properties. In the first place, it must last for a certain finite time, for if it be of shorter duration than this it will not be perceived. Secondly, it must be larger than a certain size. Thirdly, its intensity must be greater than a certain limiting quantity. Fourthly, the rays it emits must have wavelengths which lie between certain limits. So that in the case of each of these four properties there are limiting values which must be exceeded; these values are called thresholds. The retina has, distributed over its surface, two different types of sensitive organ: the cone apparatus, which has the function of perceiving colours and is used in day vision; and the rod-visual purple apparatus, which is colour blind but very sensitive to light of low intensity and therefore used for twilight vision. Owing to the fact that the distribution of these organs is not uniform, we have to state the part of the retina which is being stimulated, when assigning a value to any of the above-mentioned thresholds. For example, the threshold for intensity may be that which just actuates the rods, that is the achromatic limit, or that which is sufficient to affect the cones and therefore causes an appreciation of colour. Moreover, the value of any one threshold is to a considerable extent controlled by the value of the other factors which have been mentioned; for example, the time threshold is shorter the greater the size and intensity of the light source. The exact conditions must therefore be carefully stated in quoting the value of a threshold. Lastly, we must consider the personal equation of the observer, and also the state of his vision, for both are affected to an important extent by constitution, health, fatigue, &c.

INTENSITY THRESHOLD FOR LIGHT (ACHROMATIC). If a spectrum be gradually reduced in intensity, it loses its colour and finally appears to the eye as a bright band which has its greatest luminosity in the green region of the spectrum and gradually fades towards both the red and violet ends. Since the band is colourless, any one part may be matched by any other part by suitably adjusting the intensities. But

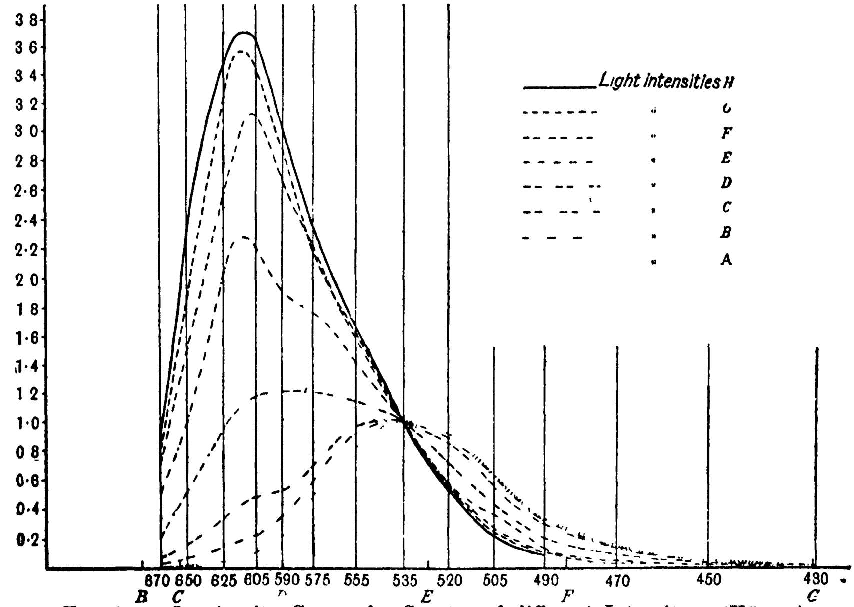

FIG. 275. Luminosity Curves for Spectra of different Intensity. (KÖNIG.)
H = highest; A = lowest; Abscissæ = wavelengths, Ordinates = luminosities. The maximum of curve for light of high intensity is seen to be at 610 $\mu\mu$, that at low intensity 535 $\mu\mu$.

compared with the appearance under ordinary intensity, the red region of the spectrum has become greatly reduced in visibility, while the blue has become relatively brighter. The part of the spectrum with maximum luminosity is found to be the yellow when the intensity is high, but to be the green when it is low. It is therefore this shifting of the position of the maximum in the spectrum which has caused red to darken and blue to become lighter. The relative forms and positions of the luminosity (apparent brightness) curves for spectra of various intensities are shown in Fig. 275.

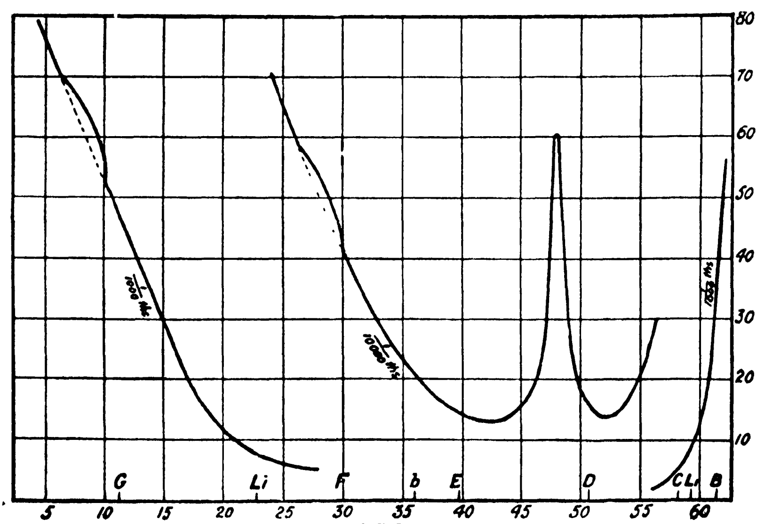

FIG. 276. 'Extinction of Colour' Curve. (ABNEY.)
Abscissæ = wavelengths; Ordinates = intensity in candle feet when colour just vanishes.

If the spectrum of low intensity be still further decreased, a point will be reached at which the different parts become invisible to the eye ; this will occur first with the ends, and last with the middle (at about 5271 A.U.). It is found, however, that the intensity values at which visibility ceases decrease the longer the eye is kept in the dark—that is to say, the retina gradually becomes 'dark adapted.' The curves obtained for different degrees of dark adaptation are also shown in Fig. 276.

We must now consider the effects on the achromatic threshold, of size of light source, duration of stimulus and part of retina illuminated. With regard to size, experiment shows that, as the size of the source decreases, so the intensity at which extinction occurs increases, in fact that the area of the source multiplied by its intensity is a constant. With regard to the region of the retina that is stimulated, it is found that the rod-visual purple apparatus is responsible for the appreciation of the light of low intensity ; it is therefore found in all parts of the retina other than the fovea centralis from which rods are absent. The effect of time of stimulus will be considered shortly.

THE DIRECTIONAL SENSITIVITY OF THE RETINA. Stiles and Crawford found that a ray of light reaching the fovea through the centre of the pupil causes a greater stimulating effect than a ray which has reached the fovea through a more peripheral part of the pupil. This effect was not caused by the lens system of the eye and must therefore be due to a property of the retina. Recently Stiles (1939) has repeated the above test using light of different wavelengths and working with both the fovea and the periphery (extra fovea). Some of his results are given in the following table, which gives the light intensities which must be employed in order that a beam of light through the periphery of the pupil shall be as bright to the retina as one passing through the centre of the pupil.

		Nasal Side by			Centre of Pupil.	Temporal Side by		
		3 mm.	2 mm.	1 mm		1 mm	2 mm.	3 mm.
Fovea (light or dark adapted)	Red	4·8	2·7	1·5	1·0	0·9	1·1	1 6
	Yellow	3·8	2·3	1·4	1·0	0·9	1·0	1·4
	Green	4·7	2·6	1·4	1·0	0·9	1·1	1·7
	Blue	5·2	3·2	1·5	1·0	0·9	1·1	1·8
Periphery (Para-fovea) (light adapted)	Red	3·8	1.9	1·2	1·0	1·1	1·6	3·0
	Yellow	2 9	1·6	1·1	1·0	1·2	1·7	3·2
	Green	2·8	1·6	1·2	1·0	1·1	1·6	2·6
	Blue	2·8	1·6	1·2	1·0	1·1	1·6	2·6
Periphery (Para-fovea) (dark adapted)	Red	2·9	1·9	1·2	1·0	1·0	1·4	2·3
	Yellow	1·3	1·1	1·0	1·0	1·0	1·1	1·3
	Green	1·0	1·0	1·0	1·0	1·0	1·0	1·0
	Blue	0·9	0·9	1·0	1·0	1·0	1·0	0·9

Thus if two beams of red light fall on the fovea, one through the centre of the pupil, the other through the periphery of the pupil 3 mm. to the nasal side of the centre of the pupil, then for the beams to appear equally bright the peripheral beam must have 4·8 times the intensity of the central one. The conclusion is that light falling obliquely on the retina is less effective than light falling at right angles to the retinal surface.

INTENSITY THRESHOLD FOR COLOUR. If a spectrum of low intensity, which appears colourless to the eye, be gradually increased in brightness, a point will be reached at which the colours begin to be

recognisable, first yellow and green, then blue and lastly red and violet. If the intensity at which the colour just vanishes is measured, the curve obtained is similar to that shown in Fig. 276. As the intensity is increased, the point of maximum luminosity gradually shifts from the green to the yellow. As light is gradually increased in intensity, an object is first seen without colour, but on further increase the colour also is recognised ; this is called the photo-chromatic interval. It follows from what we have said that the interval is greatest for colours of short wavelength (blue) and least for long (red) (the Purkinje phenomenon). The thresholds for light and colour differ in another important respect : whereas that of light varies with the degree of dark adaptation, that of colour is found by experiment to be nearly constant. With regard to the effect of area of light source, it is found that the same type of relationship exists in the case of colour as for light : that as the area is diminished, so the intensity must be correspondingly increased. The area and intensity, multiplied together, do not, however, equal a constant as they do in the case of light. The appreciation of colour is associated with the cones and is therefore most highly developed at the fovea. As the periphery of the retina is approached, the number of the cones very rapidly decreases, and we should therefore expect to find a limit to the size of the visual field for different colours. This may be tested by means of the perimeter (Fig. 269) and small coloured discs, or more accurately by suitable apparatus for employing spectral colours. By these methods it is found that the colour fields are smaller than those for light, but more or less concentric with them (Fig. 277). The actual size of the fields varies with the intensity and size of the test light source or object. The order in which the colours disappear varies somewhat, but appears to be usually as follows :—First green, then yellow, then red, and lastly blue. The determination of the size of the colour fields is a technique of considerable practical importance, because they are found to become restricted in those progressive lesions of the optic nerves which may finally lead to total blindness, and also in inflammatory conditions of the retina and choroid. Careful examination of the apparent limits of the blind spot by means of similar apparatus was found by Haycraft to show that there is a similar variation in the relative sizes of the fields for different colours (Fig. 202). The same phenomenon is also found round the blind spots formed in the retina by disease.

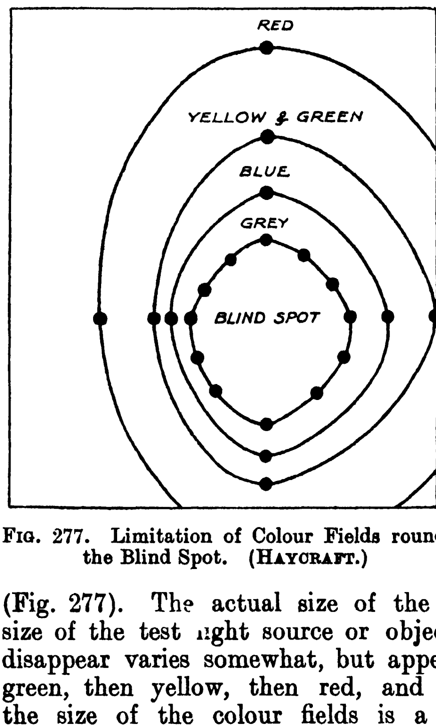

FIG. 277. Limitation of Colour Fields round the Blind Spot. (HAYCRAFT.)

SIZE THRESHOLD OR VISUAL ACUITY. If a small source of light be gradually reduced in size, a point is soon reached at which it becomes invisible. If it is a coloured source, it as a rule shows a well-marked photo-chromatic interval, that is, it first loses its colour and then disappears later. If the intensity of the source is very great, the size has to be much reduced before it becomes invisible : it is because of this that we see the

stars. If the size and intensity at the point of disappearance be measured, it is found that when multiplied together they give a constant, so that as in the case of the light threshold, the determining factor appears to be the amount of light which falls on the retina. In the case of intermittent illumination a similar relationship is found.

Visual acuity is the ability to see as separate the images of small bright light sources of any shape placed very close together. Experiment shows that the distance between the sources must be increased as their distance from the eye is increased: in other words, that the angle which they make at the eye must be greater than a certain limiting value. The angle usually obtained is an angle of one minute, and on this the test types used in practice for testing the visual acuity of patients is based (see p. 413). Persons with exceptionally good vision are able to see the separate images when the angle which the sources make at the eye is very much less than this, namely 24 seconds. Assuming that the posterior nodal point is 15·6 mm. from the retina (this being the distance in the normal eye), 24 seconds corresponds to a distance between the images at the retina of 0·0018 mm. The diameter of the cones is between 0·0020 and 0·0030 mm., and in the fovea they are very closely packed. The maximum visual acuity is therefore certainly as great as the size of the cones would lead us to suppose possible.

A dark spot on a bright ground is similar to the case just considered, because for the dark spot to be recognised it must subtend at the eye the minimal angle mentioned above. But increasing the intensity of the ground or the blackness of the spot will only make a very small difference, so that unlike the bright spot, an increase in the intensity does not make up for a difference of size.

THE DEPENDANCE OF VISUAL ACUITY ON LIGHT INTENSITY. It is well known that visual acuity is very low when the eye is used under conditions of dim illumination. Even well-defined objects appear nebulous when seen in poor light. As the illumination is increased, definition improves with very great rapidity until at great intensities of illumination very high acuity is reached. When visual acuity is plotted against illumination an S-shaped curve is usually obtained.* Some observers, *e.g.* Köenig, have reached the conclusion that visual acuity reaches a maximum value at about 100 ft. candles and that no improvement results from increasing illumination above this amount. Lythgoe, however, states that if care be taken to adapt the eye adequately, then visual acuity goes on improving as the illumination increases above 100 ft. candles. This relationship of visual acuity and intensity forms the basis of modern methods of artificial illumination, the brightness used depending on the visual acuity that is required of the observer. Thus, for passages and store-rooms 1/10 ft. candle may be sufficient. For coarse work not requiring detailed examination, 1 ft. candle may suffice. For clerical work, 10 ft. candles may be found necessary. For work where fine detail is to be seen, 100 ft. candles may be wanted, and 1,000 ft. candles will probably not be excessive for the finest work of all. Thus, for seeing very fine structures under a microscope, a bright microscope field is necessary.

THE DEPENDANCE OF THE BRIGHTNESS DIFFERENCE THRESHOLD ON LIGHT INTENSITY. The ability of the eye to detect a difference of brightness is found to change considerably with illumination. When illumination is poor, quite big differences of brightness are not noticed. As illumination improves the differences which can be perceived get

* "Foundations of Experimental Psychology," 1929, p. 246, Fig. 15.

smaller and smaller until, at very high illuminations, the differences become extremely small. If the difference, which can be detected by the eye, is plotted against the intensity, a curve* is obtained from which the conclusion is that, as in the case of visual acuity, the illumination required by an observer will depend on the differences of brightness that have to be visible to his eyes. If the differences of brightness are considerable, for example white objects lying on a black ground, a low degree of illumination will suffice. As the differences get smaller, the illumination required gets larger, so that where it is necessary to see objects which have nearly the same brightness as their surroundings then quite high intensities of illumination may be required.

Much the same statement applies to differences of colour. The perception of small colour differences is very greatly facilitated by a high degree of illumination. In consequence, for the most perfect colour matching which the eye can carry out, a high degree of illumination should be employed.

HECHT'S THEORY OF VISION. Hecht's theory is based on the duplicity theory, namely that the retinal receptors consist of both rods and cones ; the rods occupying the more peripheral parts of the retina and being excluded from the fovea ; the cones occupying the central part of the retina and having exclusive tenancy in the fovea. According to Hecht, these form two populations which differ considerably in the quantity of light required to stimulate them, the rods being, on the average, enormously more sensitive than the cones. Now according to Hecht the rod population consists of members which differ greatly in sensitiveness ; a few have relatively low sensitivity, a few have relatively high sensitivity, and between the two come the main mass of the rod population. A similar statement, according to Hecht, applies to the cones. Now these two populations overlap so that as illumination is increased from zero, and therefore more and more rods are brought into play, some cones have commenced to act before the intensity has reached such a value as to cause a response from the least sensitive of the rods. This hypothesis is advanced by Hecht in order to explain the effects of increasing illumination on visual acuity and on brightness difference threshold. Thus, at very low intensities, only a few scattered rods respond, the consequence being low visual acuity and low discrimination of difference of brightness. A small increase in illumination will bring more rods into an effective condition, visual acuity will thus improve and, since the additional rods thus brought into play do not differ from one another so much in sensitiveness as the ones stimulated by the previous low degree of illumination, the discrimination of differences of brightness will also show an improvement. As the illumination is still further increased, more and more rods act until the brightness reaches a value at which the main rod population begins to function. At this point the differences of threshold between these individual rods is small, and so the ability to perceive differences of brightness has reached a much higher value than before. Now as the illumination is still further increased the cone population begins to be affected. The number of percipient elements, therefore, increase with a corresponding increase in visual acuity and, since the cones are more nearly alike in sensitiveness than the rods, the ability to discriminate small differences of brightness is also greatly improved. Further increases in illumination bring more and more cones into play until the stage is reached at which the main cone population begins to come in, and this causes an enormous increase in visual acuity and a correspondingly large improvement in the ability of the eye to perceive small differences of brightness.

* "Foundations of Experimental Psychology," 1929, p. 254, Fig. 18.

There are three criticisms which can be advanced against this theory: (1) that it requires very high illumination indeed to cause the whole of the cone population to reach a functioning state. Which means that under ordinary conditions of illumination only a fraction of the cones are playing a part in vision. It may ultimately be found that such is actually the true state of affairs just as there may be sensory organs for pain which, for the greater part of our lives, are never called on to respond, but which are capable of functioning immediately a nocuous stimulus of adequate intensity is applied to the particular part of the body in which these pain end organs are situated. (2) That it assumes that the sensory structures of the retina obey the all or none law; that is that a rod or a cone can only be in one of two conditions, not responding at all or responding to the greatest extent of which it is capable. If the rods and cones obeyed this law they would differ from all the other sensory end organs which have been experimentally investigated. Moreover, experiments on the retina of Limulus have shown that its retinal receptors are graded in their responses. When illumination is low only a few impulses per second are transmitted to the brain by the optic nerve. As illumination increases, the number of impulses sent up the optic nerve increases.* In human beings, therefore, we should expect the impulses transmitted by the various fibres of the optic nerve to depend on the intensity of stimulation of the rods and cones to which the various fibres are attached. (3) That it does not consider cases where the visual acuity exceeds by a very material amount that which would be expected according to the law of geometrical optics. The position with regard to these cases will now be examined.

THE DIAMETER OF THE CONES OF THE FOVEA. A large number of investigators have determined the dimensions of the cones taken from the fovea centralis of the eye. They vary considerably as the following table shows. The mean value being about $3{\cdot}2\mu$.

Observer	Diameter of cones
Max Schultze	$2{\cdot}8\mu$
H. Muller	less than $3{\cdot}0\mu$
Merkel	$3{\cdot}0\mu$
Wadsworth	$2{\cdot}5\mu$
Kuhne	$2{\cdot}0$–$2{\cdot}5\mu$
Kolliker	$4{\cdot}5$–$5{\cdot}4\mu$
Koster	$4{\cdot}4\mu$
Grief	$2{\cdot}5\mu$
Dimmer	$3{\cdot}0$–$3{\cdot}5\mu$
Mean	$3{\cdot}2\mu$

If the focal length of the eye be assumed to be 15 mm., then $3{\cdot}2\mu$ corresponds to an angle of 44 seconds of arc.

THE RESOLVING POWER OF THE EYE. The values obtained for the resolving power of the eye differ according to the type of test which is used. Thus, Hooke found that two stars must be separated by approximately 60 seconds of arc in order that they may be seen separately by the eye. The distance between the bright bars of a grating, in order that resolution shall occur, have been found in general somewhat less, as shown in the following table.

* *J. Cellular & Compar. Physiol* (1), 1932, p. 277, Fig. 6 (p. 291).

Observer.	Value at retina in		Angle in seconds of arc.
Lister	4·6	...	64
Hirschmann	3·6	..	50
Bergmann	3·8	...	52
Helmholtz	4·6	...	64
Uthoff	4·0	...	56
Cobb	4·6	...	64
Mean	4·2	...	58

For some other test objects the acuity of the eye exceeds, by a considerable margin, the above values. Thus, Volkmann, in 1863, found that the smallest difference that can be perceived between the breadth of two white objects on a black ground was as little as 10 seconds of arc. Wulfling, in 1892, confirmed Volkmann's observations. Hering, in 1899, showed that the edges of two rectangles did not appear in line with one another if they were shifted sideways by as little as 10 seconds of arc. He named this ability to identify small displacements as "the position sense." Hartridge confirmed Hering's value. Bryan and Baker, 1912, found that one line of a vernier can be set into coincidence with another line of a vernier with an accuracy of between 8 and 10 seconds of arc. Hartridge found that objects with diffuse contours (like absorption bands) can be set into line with an accuracy of approximately 11 seconds of arc. It will be observed that all these experiments indicate an accuracy of observation of approximately one-sixth of the diameter of a foveal cone. Even greater accuracy is found when the acuity of vision is determined for the narrowest black line that can be perceived on a white ground. The values obtained for several observers is given in the following table.

	Seconds of arc
Aubert	6
Smith Kastner	3·5 Spider's web.
Hartridge	3·6 Bright brass wire.*
Hartridge and Owen	3·1 Black brass wire.*

Recently Hecht and Mintz, using as test object a black line whose length was many times its width, obtained values for visual acuity even better than those quoted in the above table. They obtained values as low as 0·5 seconds of arc.

The acuity of stereoscopic vision has also been found to be extremely high. Thus, Bowden found an accuracy of 5 seconds of arc, while Breton, 1920, found an even greater accuracy of 4 seconds of arc; which would correspond to an accuracy of one-fifteenth of the diameter of a foveal cone. It is clear that some theory is required which will account for the very great acuity that experiment shows that the eye possesses for these kinds of test.

HARTRIDGE'S THEORY OF VISUAL ACUITY. On the basis that the eye suffers from chromatic aberration and diffraction, Hartridge calculated the distribution of light intensity in the image formed on the human fovea. This calculation was done for (*a*) a grating test object, (*b*) for a narrow black line on a white ground, (*c*) for the coincidence of two black lines on a white ground (or two white lines on a black ground), and (*d*) for the stereoscopic sense of position. From the distribution of light thus calculated, it was possible to ascertain the average illumination falling on the cones of the retina. It was found, for example, in the case of a single black line on a white ground, that that cone which according to geometrical optics corresponds with the centre of the black line, receives 13 per cent. less light than

* Both mounted in front of white paper at such a distance that no shadow was cast.

the cones on either side of it. If, then, this cone sends impulses up the optic nerve fibre connected to it which are noticeably less in number than the impulses on the cones on either side, the existence of this narrow black line will be identifiable and its position in space can then be observed. Similar calculations for the other types of test mentioned above have given the values shown in the following table.

A. Cases in which there is a constant difference of intensity without eye movements.	
Single black line on white ground	13 per cent.
Grating	20 ,,
Double line	12 ,,
B. Cases in which there is a variation of intensity when movements of the eyes occur.	
Coincidence of two lines	22 per cent.
Position of edge of contour	32 ,,
Stereoscopic vision	12 ,,

If the figures on the table above be examined, it will be noticed that the values obtained when there is a constant difference to be observed are somewhat smaller than when with movements of the eyes a comparison has to be made between the intensity at one moment and that at another. Otherwise these values are fairly concordant if allowance is made for experimental error, for the fact that the observations of the different test objects have been made by different observers, and for the difficulty of making even approximate calculations of the intensity of light falling on the retina.

We may summarise what has been stated above as follows: for certain kinds of test object the acuity of the eye is such as to correspond to the employment of an area considerably less than that represented by the diameter of a cone. This acuity can be accounted for if the intensity of light falling on a certain cone is noticeably different to that on the cones on either side of it. The illumination on the central cone must be from 12 per cent to 32 per cent. greater or less than that on the two cones next to the central cone.

Now the question which presents itself is this: "is there any evidence that such differences of intensity can be observed?" because if they can, then the above theory is substantiated.

Now, when large retinal areas are being stimulated and the intensity of illumination is high, the smallest difference of intensity that can be perceived as given by various observers is as follows:—

Koenig	1·56 per cent.
Helmholtz	0·6 ,,
Aubert	0·68 ,,
Hartridge (with absorption bands)	about 0·5 ,,

But experiment shows that the smaller the area of retina that is being tested, the greater must be a difference in intensity for it to be perceived. For so small a retinal area as that corresponding to one or two cones it would be quite possible that the difference threshold would have fallen from the high values given in the table above to the lower values of 12 per cent. to 32 per cent. required to explain the acuity of the eye mentioned previously in this section.

Hecht and Mintz, employing a similar method of calculation, found for a black line test object a difference of illumination of 0·95 per cent. between the row of cones corresponding to the centre of the geometrical image of the black line and the rows of cones on either side of the central row.

Now all the above experiments were probably performed under approximately ideal conditions of illumination. As the intensity of illumination

decreases there will be a deterioration in the performance of the eye which is due to the fact that the ability of the eye to detect a small difference of brightness depends on adequate illumination, and falls progressively as illumination decreases.

TIME THRESHOLD. In considering the time threshold, two different sets of conditions require description: firstly, the minimum time during which a given stimulus must act in order to reach consciousness, and secondly, the minimum rate at which a series of stimuli must follow one another in order to give a uniform impression without flicker. Both are of considerable importance, since the first enters into such problems as the determination of the length of time during which a lighthouse beam should be caused to travel in a given direction; the second because it gives a reliable method of comparing the intensity of lights of different colour. Experimental investigation of the first type of time threshold is effected by measuring the length of stimulus necessary to cause a source of a certain intensity to affect the retina; and it is found that the lower the intensity the longer must the image fall on the retina. But if the eye be dark adapted, if the time and the intensity values be multiplied together, then within limits a constant is obtained. On the other hand, in the light adapted eye, the value is found to vary somewhat, but is sufficiently constant to show that the relation between intensity and time is approximately the same. Within limits, therefore, we find that at the threshold the total amount of light is constant, whether it be of high intensity for a very short period of time, or of low intensity for a correspondingly longer one. This relationship ceases to be true if the time of stimulation is longer than about one-tenth of a second, and this is apparently due to the fact that the retinal apparatus reaches a steady state in about one-quarter of a second in the dark adapted eye (rod-visual purple apparatus). A lighthouse flash should therefore be visible to the eye for this length of time, in order to make the greatest possible impression. For coloured lights, approximately the same values are found, provided that allowance is made for the comparative intensity of the colour. Since the time required for the retina to reach a steady state is nearly that at which a series of stimuli must fall on the retina in order to produce a uniform sensation, for intensities near the threshold the rate at which flicker disappears is one stimulus every quarter of a second. But it is found that as the intensity rises, the rate must be increased in order to abolish flicker. The rule which most nearly expresses the relation appears to be that geometrical increase in the intensity requires an arithmetical increase in the rate. Sherrington showed, however, that the results are affected by simultaneous contrast. This phenomenon of flicker, as has been said, is used in practice for measuring the intensities of light sources. Two methods are employed: firstly, that in which the two light sources to be compared are measured separately for the intensity at which flicker ceases when the same rate of stimulation is used for both; and secondly, that in which the two sources are caused to fall alternately on the eye, and are adjusted in intensity until flicker ceases. Of the two methods, the latter is the more accurate. The value of these methods lies in the fact that they measure brightness independently of colour. The shape of the curves obtained by plotting the luminosity of different parts of the spectrum has been shown in Fig. 276.

Lastly, we have to consider the relationship between time, intensity and apparent brightness, in the case of an intermittent source, the rate of which is sufficiently great to avoid flicker. Experiment shows that the brightness increases in proportion as either the intensity or the time intervals are

increased, and further that equal brightness is obtained if the time multiplied by the intensity is constant. This statement is true of both the cone and the rod apparatus, and is known as the Talbot Plateau Law. Use is made of this law in the sector method of controlling intensity (see p. 203) because the intensity is proportional to the time during which the light is allowed to pass through, which is in its turn controlled by the angle between the blades of the sector.

COLOUR THRESHOLD. On testing the violet end with a photographic process, or the red end by a thermopile, it can readily be shown that the spectrum extends at both ends far beyond the visible limit. The visible limit at the red end under the most favourable conditions has been found to be 8350 A.U., while under ordinary circumstances it is difficult to go beyond 8000 A.U. Since rays beyond this reach the retina in considerable amount, the limit cannot be caused by opacity of the eye media, and must therefore be due to an actual inability on the part of the retina to record their presence.

Of several hypotheses which might be advanced for this inability, the most probable is that the retinal pigments are unable to absorb rays in the infra-red part of the spectrum, and therefore, according to Draper's law, such rays cannot produce photochemical change and cannot be perceived by the eye. It may be mentioned in this connection that few organic pigments absorb strongly in the infra-red.

The limit at the violet end is less easy to determine, because the eye media, in common with a large number of other bodies, have the property of fluorescing when the ultra-violet rays fall on them, *i.e.* they convert them into rays of longer wavelength and therefore make them visible. The resulting impression is, however, quite different, because, since these rays are generated in the eye media themselves, they are spread over the retina as a haze without there being any proper image formation. The limit of the visibility of the violet end of the spectrum appears to be at about 3800 A.U., while the portion which is seen because of the fluorescence, which it produces, and which appears a pale lavender, ends at about 3200 A.U. Since the wavelength of the extreme red rays is a little more than double that of the extreme violet, the eye is sensitive to a little over an octave. The range of appreciation of the eye is therefore very much smaller than that of the ear, which is about 10 octaves. As age increases, the eye media become yellow in colour; this change particularly affecting the lens, the violet end of the spectrum becomes shortened owing to absorption. On removing the lens of the eye, as in an operation for cataract, the sensitiveness to the violet end of the spectrum is considerably increased. It would therefore seem certain that the limitation of the spectrum at the violet end is largely due to absorption by the eye media, and not to inappreciation on the part of the retina. The causes of the limitation of the two ends of the spectrum are therefore different.

DIFFERENCE THRESHOLDS. Beside the thresholds for light, colour, time and wavelength, which may be called absolute thresholds, there are certain difference thresholds that must be considered. Thus, for example, a certain finite difference must exist between the intensities of two sources of light of the same colour for a difference between them to be appreciated by the eye. There are four principal types of difference threshold: that of intensity, that of colour, that of saturation, and that of size.

DIFFERENCE THRESHOLD OF INTENSITY. It is found by experiment that a just perceptible difference between the intensities of two surfaces varies with the mean value of their intensities. Thus, supposing that it had been found by one experiment that a difference of intensity of one foot candle was necessary

in order that two sources should be just distinguishable, the average intensities of which were one hundred foot candles, then in another case in which the average was 25 F.C. the least perceptible difference would be found to be one-quarter F.C. This is one illustration of the principle known as Weber's law. It appears to be true for light of medium intensity, and for sources not separated by more than a small interval. But the least perceptible difference is found by most observers to be less than that taken for purposes of illustration above, namely one-hundreth part of the mean intensity; thus Helmholtz found it to be a $\frac{1}{167}$th, other observers have obtained even smaller fractions. It is interesting to find that the results are not influenced by the size of the pupil.

DIFFERENCE THRESHOLD OF COLOUR. If the range of colours exhibited in the spectrum be carefully examined, it will be seen that there are certain parts, notably at the red and violet ends, at which the change of colour with wavelength is a very gradual one. At other parts, on the contrary, the change of hue is very rapid; the yellow region at 5800 A.U. may be given as example. If therefore we determine by experiment what difference of wavelength is just perceivable by the eye, we find that it varies with the part of the spectrum under observation. We may therefore conveniently express the difference threshold in different parts of the spectrum in the form of a curve, as in Fig. 278. In persons with normal vision the total number of different

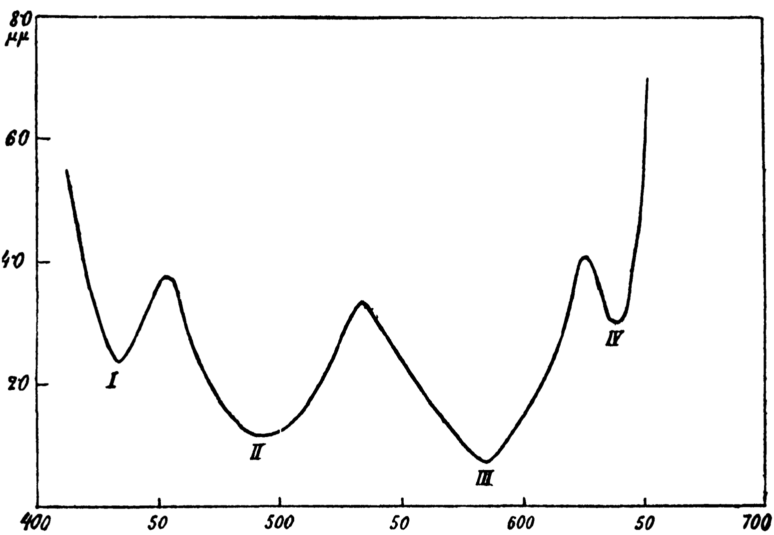

FIG. 278. Curve showing Difference Threshold for Colour at different parts of the Spectrum.
Abscissæ = wavelengths. Ordinates = difference between wavelengths capable of being discriminated. (STEINDLER.)

hues in the spectrum is calculated to be 165. In persons with colour blindness the number is greatly reduced.

This fact has been applied by Edridge Green for the detection of colour blindness; details of the method will be given later. It should be pointed out that, since in this method the spectrum itself is presented to the observer, so that there is a gradual change from one colour to the next, it is the threshold of rate of change of colour that is determined, and not difference threshold of colour. Edridge Green's method gives only from 21 to 28 monochromatic patches in the spectrum.

Difference Threshold of Saturation. By saturation is meant the amount of white light which is present with, and is therefore diluting, a colour. The threshold would appear to be of the same order as that of intensity given above, namely that a difference in the amount of white light diluting a colour by $\frac{1}{100}$th of the total intensity present can be just appreciated by the eye.

Difference Threshold of Size. If two objects are the same distance from the eye, and are close to one another and in similar positions, a difference of one-hundredth the mean size can, as a rule, be appreciated. If they are at different distances from the eye, or are far apart, or are not in similar position (*e.g.* one perpendicular and the other horizontal), then considerable errors may occur.

The Method of Comparisons. In the application of this method three different series of investigations have been carried out: (1) To determine the intensities of three primary colours which, when mixed together, will match the different spectral colours or white light. (2) To determine the intensities and wavelengths of the complementary colours. (3) To ascertain the intensities and wavelengths of red and green rays which, when mixed together, match a pure spectral yellow.

The colour box in some form or other is used for these tests. Abney's apparatus may be described as a typical example. Light from an arc lamp is focussed on to the slit of a powerful spectroscope, which consists of a collimator, a train of prisms and a telescope. The spectrum thus produced is caused to fall on three slits, one of which

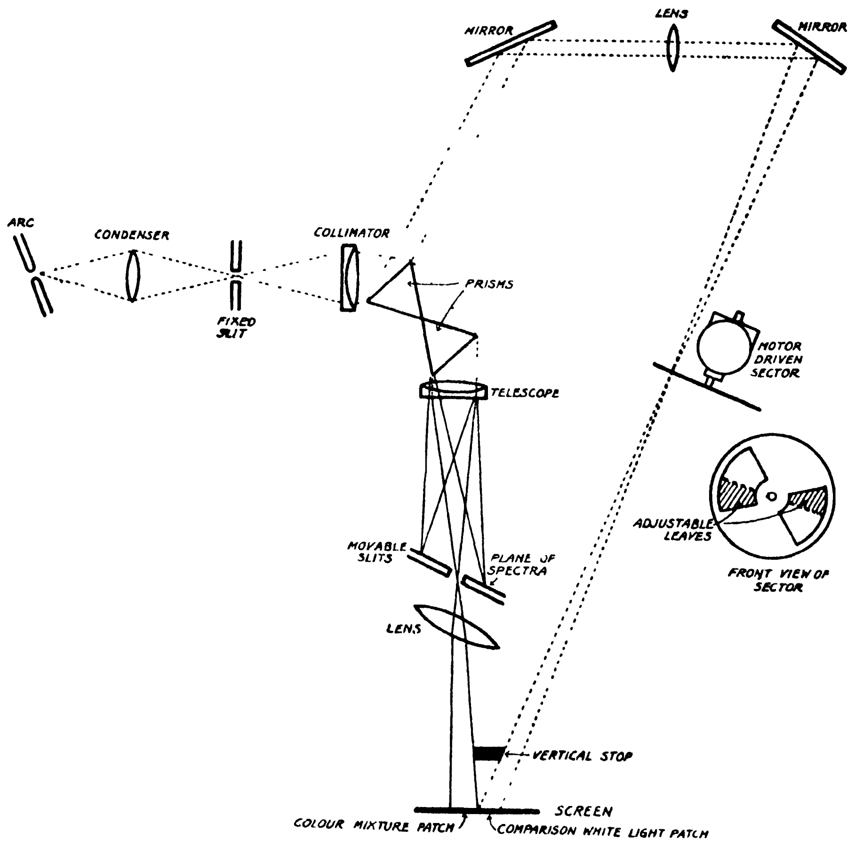

Fig. 279. Colour Patch Apparatus for Mixture and Comparison of pure Spectral Colours. (Abney.)

corresponds with the red, another with the green, and the third with the blue. The light having passed through the slits falls on a lens which forms an image of the prism faces on a screen. The light from the slits thus recombines on the screen to produce a bright patch, the colour of which alters according to the intensities of the three components. To one side of the patch a second patch of light can be thrown from the arc lamp, and this also may be varied in different ways according to the nature of the experiment. The intensities of the different beams could be modified by altering the widths of the slits ; a preferable method is to employ rotating sectors, the angles between the blades of which can be varied at will (see Fig. 279).

Colour mixing experiments performed with this apparatus give results that have already been briefly considered in Section I. It is found that not only do the three primary colours, when mixed together in the right proportions, form a white light that is indistinguishable from ordinary white light, but they can also be made to match the whole range of colours both of the spectrum and of pigments. It was also described how that certain pairs of colours when mixed in the right proportions are able to match white, and that these pairs are called complementary colours. If the colours that are mixed are further apart than are the complementary colours, then the mixed colour is found to be a shade of purple ; but if nearer together than the complementaries, then the colour formed by the mixture corresponds to an intermediate part of the spectrum. Thus, if the colours mixed are red and green, the intermediate yellow and orange portions of the spectrum can be matched. As a rule the mixed colour is not so pure as the corresponding spectral colour, being less saturated (that is, diluted with a certain amount of white light). The mixture of red and green is an exception, because it is found that accurate matches with spectral yellow can be made if the green component be not shorter in wavelength than 5400 A.U. These facts can be expressed diagrammatically in the form of a geometrical figure, the colour triangle, in which the three fundamental colours occupy

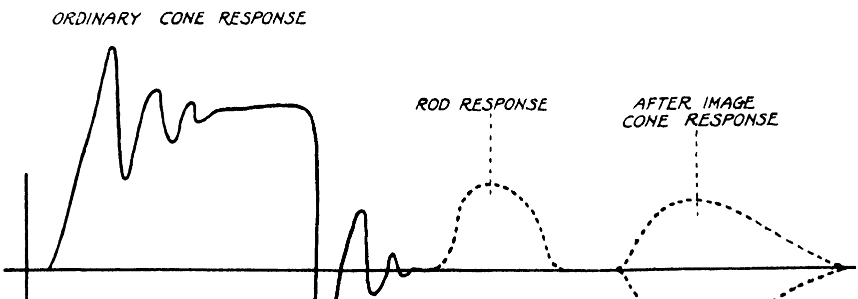

FIG. 280. Curve representing diagrammatically the Sensations aroused when the Eye has been Stimulated by a Flash of Light.
Intensity of Sensation vertical. Time horizontal. (HARTRIDGE.)

the corners and white the centre (see p. 388). Matches made by light of one intensity require readjustment if the intensity be changed, and matches made by one observer are different from those made by another. The variation with intensity is readily explained by the shifting of the centre of the luminosity curve from the yellow towards the green as the intensity is lowered. The amount of red required in a match will become increasingly greater, and that of the blue less, as the intensity is lowered. The variation with the observer, when small, is explained by individual peculiarity in the pigmentation of the eye media or of the fovea centralis ; but when considerable, by abnormality in the response of the retina to colours. Because of

this the method of colour mixtures forms a very valuable technique for the investigation of colour blindness.

THE FLICKER METHOD. The majority of observers can obtain consistent measurements of intensity with the above method only when the colours of the two patches are exactly alike. Thus it is difficult to adjust a green light to be of equal apparent brightness (luminosity) with a red because the difference in colour makes the judgment of brightness inaccurate. Abney found that in his own case practice greatly increased the certainty with which the measurement could be made. A more reliable technique is given by the flicker method. The two patches are viewed through a rotating sector, the speed of rotation of which can be controlled. The intensity of one of them is now adjusted so that, when the speed of the sector is altered, both commence to show and to cease showing flicker at the same time. By applying this method to the colour mixing apparatus, the luminosity of the different parts of the spectrum can be determined. The curves obtained at different luminosities have already been given in Fig. 278. Tested by this method, different observers show individual peculiarities, which amount in some cases to a greatly diminished perception of a certain part of the spectrum. Some of the types met with will be described later.

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8. THE SUBJECTIVE PHENOMENA OF VISION

By ' subjective ' we mean that the sensations under consideration cannot be traced solely to the stimulus which initiated them. Thus intermittent stimuli presented to the eye at a certain rate form a continuous sensation, so that flicker appears to have ceased. But the carrying on of the sensation from one stimulus to the next is performed by some part of the visual mechanism, and has nothing to do with any physical peculiarity of the light, It is, therefore, an example of a subjective phenomenon.

THE SENSATION CURVE. When a light stimulus enters the eye a certain period of time elapses before a sensation is perceived. This latent period may be compared with that which occurs between the stimulus and contraction of a muscle. After its commencement the sensation rapidly rises to a maximum (see Fig. 280) and then shows several rapid fluctuations as it reaches its mean value. These fluctuations are called Charpentier's bands (Fig. 281), and are well seen after stimulating the eye by means of the flash from an electric spark. They have been compared with the oscillations which occur when an electric current is passed down a telegraph cable, and which are caused by the inductance and capacity of the circuit. Similar oscillations occur when the telegraph circuit is broken. Oscillations have also been witnessed and described by Bidwell at the end of the primary visual response. The time taken for the sensation to reach its maximum varies from 0·16 to 0·07 secs., being shorter the greater the intensity of the stimulus. This so-called primary image is followed under certain conditions by a less definite and less intense image, which has the following characteristics: (1) It is not seen when the eye is light adapted. (2) It is strongest for green light, and is absent

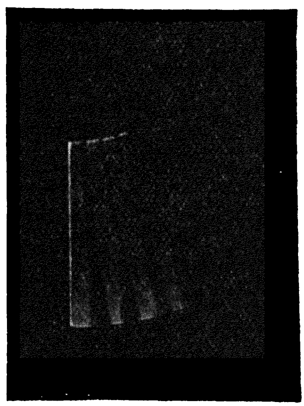

FIG. 281. Charpentier's Bands as seen when a disc with a narrow radial slit is rotated in front of an illuminated screen about once a second.

for red. (3) It is absent from the fovea. (4) It is not seen by persons suffering from night blindness. (5) It is always of a bluish-grey colour. All the above facts fit in with the view that, whereas the primary response corresponds with the reaction of the cones to the stimulus, the image which is sometimes seen to follow belongs to the rod apparatus.

THE AFTER IMAGE. Following these responses of the cone and rod end organs is the so-called secondary image, which certainly concerns the cones and may concern the rods as well. This image is of longer duration than those already considered, and it is of much lower intensity. It has, however, the peculiarity that, so long as it lasts, the part of the retina affected gives a diminished response to a stimulus of the same type as that which it had previously received. For example, if the first stimulus was one of white light, then a second white light stimulus falling in the period occupied by the after image of the first would not be recorded by the retina with the normal intensity. It has been pointed out that the after image period in some ways resembles the refractory period which follows the activity of muscle and nerve. If the second stimulus affects a larger area of the retina than that affected by the first stimulus, then a dark patch is seen lying on a bright ground, the dark patch corresponding in size to the first stimulus, the bright ground corresponding in size to the second stimulus. If the first stimulus is coloured, and no stimulus follows, the secondary image is found to have the same colour; but if a second stimulus of the same colour, but rather larger size, falls on the retina during the secondary image, then, as before, a dark patch is seen lying on a bright coloured ground. If, on the other hand, the first stimulus be coloured light, say red, and the second stimulus be one of white light, the sensation received is one of the complementary colour, the reason being that the red constituents of the white light are partially excluded by the after image of the first stimulus, but not so the other spectral colours, and the image which is seen is therefore a blue-green one. Because of these peculiar properties the after image is said to have two phases: being called positive when the eye receives no second stimulus, and the appearance of the after image is the same as that of the first stimulus; and being called negative when, owing to the incidence of a second stimulus, the after image shows the opposite intensity or colour to the stimulus which originated it. Since absence of the second stimulus causes the after image to be positive and the presence of a second stimulus makes it appear negative, we should expect a second stimulus of the right intensity to cause the after image to disappear altogether, since it would stimulate the surrounding retina with the same intensity as does the after image of the first stimulus. Experiment shows that this result can be achieved. Because of the importance of these properties of the after image, we may with advantage recapitulate as follows:—As a result of a stimulus the region of the retina affected gives a response which is followed by a second or after image. During this after image, this area is incapable of reacting with the normal intensity to a like stimulus, but shows increased excitability to a stimulus of the opposite kind. For example, after a green stimulus the retina is unable to respond fully to another green stimulus unless it falls either before or after the period of the after image. Therefore, if during that period a white stimulus be arranged to fall on the retina, it will cause a purple sensation (purple being white minus green) in that part of the retina first stimulated. The duration of the after image is variable, but is found to correspond roughly with the intensity and duration of the stimulus. Thus, after a few seconds' exposure to a bright light the after image may be

noticeable for two or three minutes, its intensity waxing and waning in an irregular manner. Successive images are often found to show a series of colours; a common series is bluish-green, violet, rose, and finally orange or green. The phenomenon is, however, very variable. The colours may be explained by assuming a difference in the rate of oscillation for the after images of the different colours. For example, the above series of colours would be obtained if green were more rapidly, and red less rapidly, perceived than blue.

FLICKER AND VISUAL PERSISTENCE. A study of the characteristics of the sensation curve provides an explanation of a number of the subjective phenomena of vision. For example, if a cardboard disc, marked as shown in Fig. 282, be caused to rotate slowly, while the black and white sectors are readily recognised, their radial margins appear blurred. This blurring is due to the slow rise and fall of the primary image of the sensation curve. If the speed be increased, a point is reached at which the disc gives an unpleasant glittering appearance; this would appear to be due to one stimulus occurring during the after image of the previous one, and thus becoming suppressed, but being followed in its turn by a fresh stimulus which is caused by contrast (see later) to have a greatly increased intensity. If the rate of rotation of the disc be still further increased, a point is reached at which a stimulus falls during the primary image of the previous one. The persistence of the primary image after the cessation of the stimulus causes the stimuli to fuse to give a uniform sensation without flicker, which may be compared with the complete tetanus of a muscle. Since the primary response is more abrupt the greater the intensity of the stimulus, a more rapid rate of rotation is required to produce fusion at high intensities than at low.

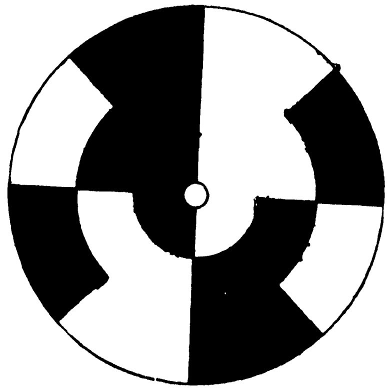

FIG. 282.

PERIODIC STIMULI. We have seen that, if a stimulus falls during the after image of a previous one, its character is altered. If the two stimuli are similar the second stimulus tends to be suppressed, but if dissimilar the second stimulus appears to be increased. If, on the other hand, the second stimulus falls either before or after the after image, it appears to be unaffected. But the experiments on flicker show us more than this, because even in a case where a second stimulus comes before the after image of the first, it is clear that the third would at a certain speed coincide with it, and should therefore be modified. Experiment seems to show no evidence for such an effect, and we must therefore conclude that the occurrence of the second stimulus in some way inhibits the after image of the first, so that its results are not apparent. Further evidence for this view is to be obtained from continuous stimuli, for we do not find a sudden diminution in the intensity of the response a moment or two after a continuous stimulus has begun, such as we should expect if the after image of the commencement of stimulus were suddenly after a short interval to assert itself. What happens to these suppressed after images? Are they entirely destroyed, or are they caused to accumulate until the end of the stimulus? The evidence appears to be in favour of the latter view, because an after image has a more definite character the longer

the stimulus. Moreover if the gaze be directed towards a fixation point, and the inclination to blink be rigidly suppressed, after a few seconds the images of objects which fall on the periphery of the retina begin to appear milky, particularly in the shadows. At the same time the brightness of the high lights seems to be reduced so that it approximates more and more closely to the milkiness of the shadows. When this stage is reached, objects appear in outline, the contours being produced and renewed by imperfect fixation. If fixation can be retained for a short period, it will be found that the whole field becomes blank with the exception of the fixation mark. If this also disappears momentarily, then fixation is lost, the eye makes an involuntary movement, and the whole field immediately fills with detail again. In this experiment, two processes seem to be going on : firstly, in the shadows, the disappearance of the after images of previous impressions, the replacement of the visual purple previously bleached, and possibly also the recovery of these parts of the retina from the effects of previous stimulation, all of which will increase the sensitiveness of the retina so that it now responds to the light reflected by the shadows ; secondly, in the high lights, the accumulation of after images, the bleaching of the visual purple, and possibly the effects of fatigue, all of which tend to reduce the intensity of the impression. So that these processes, tending to increase the brightness in the shadows, and to decrease that of the high lights, finally bring them to the same level. In these processes the accumulation and removal of after images would appear to take a considerable part. The conclusion to which we are forced is, that at the beginning of a continuous stimulus, the after images are effectively removed until such time as the stimulus shall cease, when they can be permitted to assert themselves. But if the stimulus be prolonged, the suppression becomes more and more difficult, until the accumulation of after images is so extensive that they begin to obtrude more and more on the impression conveyed to consciousness.

Fate of After Images. If the conclusion drawn from the above experiments is valid, the question arises as to the apparent unimportance of the after image in ordinary vision. The answer is to be obtained from experiments like the following :—If fixation be continued until the images formed on the retina appear in outline as in the previous experiments, and the gaze be then quickly turned to a second fixation mark placed some distance from the first, it is found that, on returning to the first mark, some time has to elapse before the appearance in outline is obtained again ; in fact the time taken is not very different from that required to reach this stage at the beginning of a new experiment. The second impression had effected an almost complete removal of the after image of the first, so that on returning to the first again, the slate had, as it were, been wiped clean, and the first impression therefore acted as if it were a new one. This conclusion is entirely in agreement with our previous conclusions with regard to the after image, namely that it corresponds to a period in which a stimulus, similar to that to which the after image belongs, is inhibited, while that of a different kind is favoured. If, therefore, during fixation the gaze be momentarily directed elsewhere, the after image that had been set up is quenched by the new impression, and on returning the gaze to the fixation point the old image behaves almost like a new one. The non-intrusion of the after image in ordinary vision is therefore due to a considerable extent to the continual and rapid replacement of one impression by another, by the shifting of the gaze, and also to the spreading of the accumulation of partially effaced after images more or less uniformly over the retina. It has been suggested that impulses

may be originated from the external eye muscles on movement, which on reaching the brain assist in the removal of the after images of previous stimulation.

ADAPTATION. If the eye after being in the dark is rapidly exposed to the light, at first the sight is confused and the eye dazzled, in spite of the powerful constriction of the pupils. The eye, however, very quickly becomes accustomed to the greater intensity, or, as we say, it becomes light adapted. In a similar manner, on entering the dark from the light, the eye can at first see nothing, but by degrees it becomes accustomed to the new conditions, and objects begin to be recognised : the eye has therefore become dark adapted. In the first case, the initial light stimulus reduced the sensitiveness of the eye to light to such an extent that the eye ceased to react to an excessive degree as it had done at first. Dark adaptation appears to consist of two separate processes : (1) the removal of after images from the cone light-receiving mechanism, and (2) the replacement of visual purple for the rod apparatus; the former predominating at high intensities and the latter at low.

FATIGUE OF THE RETINA. If the eye has been exposed to a very bright light for a considerable time, there is at first inability to see with the dazzled part of the retina. If a field is looked at, a black spot appears to lie in front of it; if, on the other hand, the field subsequently looked at is dark, this same area of the retina appears to be filled by a bright haze. If the dazzling light be restricted to one colour, there is an inability to see the same colour, if of lower intensity, immediately afterwards. The power to see other colours is apparently quite unaffected ; in fact Burch stated that the complementary colour actually appears to be more vivid than usual. These changes are similar to those caused by after images. The negative after image causes diminished appreciation of colours similar to itself, while the positive shows itself as a bright image similar in colour to the original stimulus when a dark field is looked at.

CONTRAST PHENOMENA

SUCCESSIVE CONTRAST. Visual impressions are affected by the previous history of the retina ; thus, after the eyes have been directed towards a red surface, a grey surface appears to be tinted green, a green surface seems a more vivid colour than normal, while a red colour is relatively dull. In other words, after stimulation by one kind of source, another of a similar nature is inhibited, while that of a different nature is either unaffected or may be even increased. This effect is called successive contrast. Experiment shows that the change of the second stimulus is such that it favours the colour complementary to the first stimulus. These effects are similar to those already described under adaptation and fatigue, and the causation of the phenomenon is the same as that given above, being due to the presence of an after image.

SIMULTANEOUS CONTRAST. If the retina is stimulated by two separate impressions, any differences between the impressions will be found to be accentuated. Thus if a small grey surface be placed on a white ground, the grey will become darker, and to a less extent the white ground will become lighter. If now the same grey surface be placed on a dark ground, it will be found to become lighter and the field darker. The nearer the surfaces are together the greater are the effects of contrast, the edges showing the effects of contrast most. In the case of colours, similar changes take place : thus two similar colours of different intensity placed together appear to be more different; two colours of different saturation change, the one to

greater, the other to less saturation ; while two colours of different wavelength appear, under the influence of contrast, to suffer a variation towards the complementary colour. A similar change is observed if contrast is occurring between a colour and a grey surface of approximately the same intensity, for we find that the grey is obviously tinted with a colour that is very nearly the complementary of the colour in question. It has been stated that the light which reaches the eye other than through the pupil (*e.g.* the sclera), and which is coloured an orange-pink in consequence of its partial absorption by the blood pigment in the capillaries, is responsible for the contrast colours not being strictly complementary to those which produce them. It is also found that separation of the surfaces, or the demarcation of the junction of the two surfaces by means of a narrow black or white line, or even the existence of small marks or creases, reduces the effects of contrast to a considerable extent.

We can summarise the effects of simultaneous contrast in the following way :—if one part of the retina is being stimulated, the part surrounding it not only tends to discourage a similar stimulus but also favours one of a complementary nature (black in this connection being considered as complementary to white). But this statement is similar to that which we have already made with regard to the effects of the after image on the same part of the retina which has received stimulation. Simultaneous contrast would therefore appear to be simply an extension of the after image phenomenon into a region of the retina outside the confines of the original stimulus. Experiment shows that this extension does not go far from the excited area, for the contrast effects, which may be considerable near the contour, rapidly decrease as the distance from the contour increases.

BIDWELL'S EXPERIMENT. If a brief white light stimulus be caused to fall during the after image period of a previous red stimulus, we should expect the white light to be tinted blue-green, because red light is suppressed and its complementary increased. If the intensity and time intervals are carefully chosen, the blue-green sensation may be made stronger than the original red stimulus to which it owes its colour. The persistence of vision causes this blue-green response to last an appreciable time, and therefore if another red stimulus rapidly succeeds the white one (the one which is coloured blue-green) it will tend to be suppressed from consciousness. But a white stimulus succeeding this second red one will be coloured blue-green by its after image, because it has left its impression on the visual mechanism, although that impulse has not been conveyed to consciousness. If therefore a series of such red and white impulses be caused to fall on the retina, each white one will be tinted blue-green, and each red one will be suppressed, the result being that the complementary colour alone is seen. This interesting phenomenon was discovered by Bidwell. He took a disc of tin plate about 8 inches in diameter,

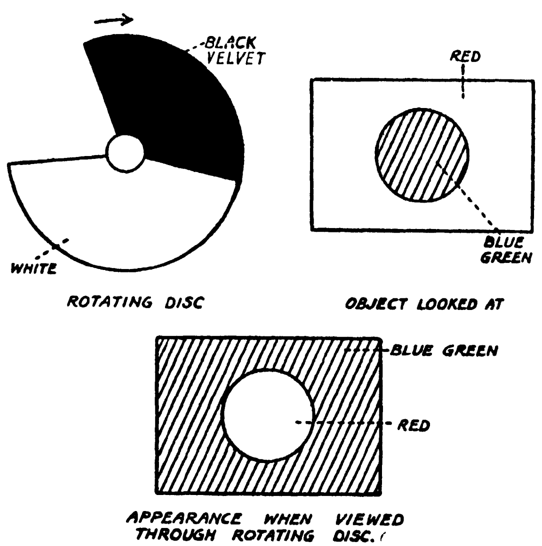

FIG. 283. Bidwell's Rotating Disc.
An object looked at through the disc appears in complementary colours.

and arranged so that it could be rotated by an electric motor 6 to 8 times a second. From this disc a sector was cut of approximately 60 degrees; half the remainder was covered with black velvet and the other half with white paper. Behind the disc were mounted two pieces of silk: one red, the other blue-green (Fig. 283). The order in which the images were presented to the eye on rotation of the disc was: (1) coloured silks, (2) white sector, (3) black sector, and so on. The result was found to be that the red silk appeared pale blue-green, and the other pink; that is, in both cases the complementary colour alone was seen. This experiment brings out very clearly the fact that the after image process is entirely subconscious. The following observations confirm this conclusion.

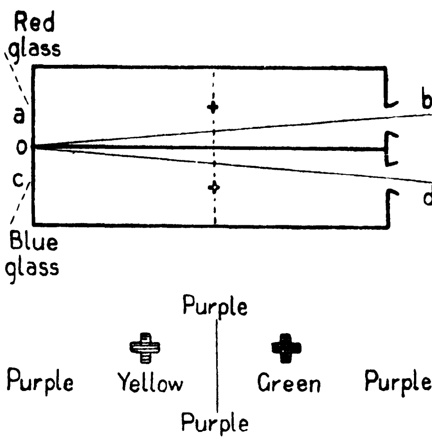

FIG. 284.

BURCH'S EXPERIMENT. It has been much discussed in the past whether contrast phenomena are due to errors of judgment, as Helmholtz supposed, or due to physical or physiological changes taking place in the retina or in the conducting paths leading from it to consciousness. Burch disproved Helmholtz' view by the following simple experiment. A box (Fig. 284) is divided into two long compartments, *a b* and *c d*. At *a* the compartment is closed by a red glass-plate and at *c* by a blue glass-plate. Apertures are provided at *b* and *d* for the observer's eyes. At + and + two small grey crosses are fixed about the middle of the compartment on sheets of transparent glass. On looking through the openings *b* and *d* and converging the eyeballs, so as to fix the point *o*, we get a fusion, more or less complete, of the two colours red and blue, so that the background appears purple; or there may be a struggle between the colours, at one time blue, at another red predominating. To the judgment, however, there is one background and not two, and therefore, according to the theory of Helmholtz, the grey crosses should by contrast both acquire the same induced colour, which would be complementary for purple. But it is found that the two crosses are perfectly distinct in colour, that which is seen by the eye against the blue ground being yellow, while that on the red ground is green, showing that the phenomena of simultaneous contrast are not due to an error of judgment.

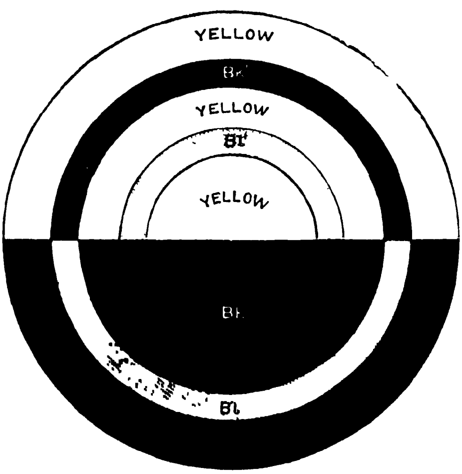

FIG. 285.

SHERRINGTON'S EXPERIMENT. The same fact is very definitely established by the following experiment devised by Sherrington. The disc (Fig. 285) presents two rings, each half-blue and half-black. The outer ring is intensified when at rest by simultaneous contrast, the black half being seen against the surrounding yellow, while the luminosity of the blue half is increased by the effect of the surrounding black. In the inner ring the blue half is darkened by contrast with the surrounding yellow, while the black half is not evident at all. If the disc be rotated, we get two concentric rings on an apparently homogeneous field. It is found however that the outer ring flickers long after complete fusion has taken place in the inner ring, showing that the stimulation of the retina by the outer ring is increased under the influence of contrast.

ALLEN'S MONOCULAR EXPERIMENTS. By means of two independent monochromatic illuminators, Allen illuminated one eye with a small flickering light source of one colour lying on a background of a large non-flickering source of another. He varied the rate of flicker until a smooth sensation was perceived. He then made the justifiable assumption that, when an increase in flicker rate is required for smoothness, it demonstrates an increase in the sensitiveness of the retina at that point and *vice versâ*. Allen found that a red background caused a decreased sensitiveness of the retina to red, but an increased sensitiveness for green and blue. A green background on the other hand made green minus, but red and blue plus, while a monochromatic yellow background made both red and green minus, but blue plus plus. Now these results fit in exactly with older ones on simultaneous contrast. They show, however, more than these could do, namely that both facilitation and inhibition are at work. They show, moreover, a trichromatic subdivision of the spectrum such as Young's theory of colour vision postulates.

ALLEN'S BINOCULAR EXPERIMENTS. By a similar arrangement to that used above, the constant coloured field was presented to one eye, and the small flickering coloured source of light to the other eye. These experiments also demonstrate a trichromatic subdivision of the spectrum, since a stimulus of red, green or blue to one eye enhances the perception to the whole spectrum, but especially to the corresponding colour in the other eye; there being two invariable regions between these at 0·655 and 0·500 μ respectively.

CAUSE OF AFTER IMAGE. Sherrington has shown that in the case of muscles there is what is called reciprocal innervation. Thus stimulation of the cortex which causes the contraction of one muscle also brings about a corresponding relaxation of its antagonist, in order that rapid and economical motion may take place. The contraction and corresponding relaxation are therefore analogous to the response of one part of the retina, which is accompanied by an inhibition of the surrounding parts of the retina to the same kind of stimulus (simultaneous contrast). In a similar manner the inclination to extension, which is found to accompany the prolonged flexion of a limb, finds its analogy in the phenomena of after images and adaptation of the eye, since the tendency is to suppress a similar stimulus in the part of the retina stimulated and to encourage its complementary. The inference to be drawn from these analogies is that the after image and its allied phenomena are caused by changes in the conducting paths of visual impressions which are similar to those found to exist in paths belonging to the motor system. What the nature of these changes in the conducting paths may be is at the present time undecided. McDougall has suggested that they are fatigue effects in the synapses of the higher conducting paths. If this view is correct, it would seem difficult to explain why after images and contrast phenomena are best seen with a rested eye.

UTILITY OF THE AFTER IMAGE. We have seen that the effect of the after image is to inhibit the possible reception of a second stimulus, and at the same time to favour the reception of one of a different nature. The process is therefore one which favours change, for not only is there a tendency to efface an old impression but also to welcome a new one. Such effects must be of great value to an organ such as the eye, the function of which to a considerable extent in everyday life is to present to consciousness the greatest number of impressions in a given time. For example, by measuring the time taken to read a passage in which almost every word was of importance, it was found that on an average eight words were read in each second, and that the eight words had an average of forty letters. It is clear from this that from eight to forty different impressions must be presented to consciousness in each second. The function of the after image in preparing the retina for the reception of new impressions would therefore appear to be a very important one. The effects of simultaneous contrast are equally important to vision, because the changes produced by it are such that the images falling on contiguous portions of the retina are made as unlike as

possible. Not only are the intensity and colour of adjacent parts of the image made more definite (this process being comparable to the effects of intensification on a photograph), but the blurring at the edges of contours, due to imperfections in the image formed on the retina, is also largely eliminated (this comparing with retouching in photography).

BINOCULAR RIVALRY must be briefly referred to here, because of the similarity which it shows with the phenomena described above. If for any reason the images formed on the retinæ have dissimilar contours, rivalry ensues, first one image and then the other reaching consciousness. This process usually occurs independently in different parts of the field, so that the visual impression consists of a patchwork of the two images (Fig. 286). Seldom, if ever, are both images seen in the same part of the retina at the same time. It is found that a number of factors can cause one image totally to suppress the other: these are interest, novelty and brightness. The importance of this suppression can be appreciated by picturing the confusion which would occur if two different images were simultaneously presented to consciousness, as would happen in animals in which different

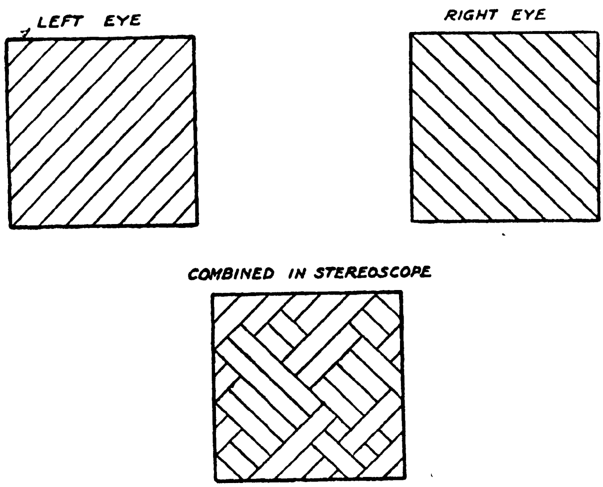

FIG. 286. Diagram to show how the Binocular Combination of two Dissimilar Images produces a Fluctuating Image containing parts of both of them.

images are formed in the two retinæ and in cases of strabismus in man. The parallelism between this process and those which we have already described can be traced by regarding for one moment one of the images as the primary one. This causes impulses to travel to the visual centres unaffected at first by the effects of other images, but these tend to accumulate more and more until the primary image is overcome, and that of the other eye put in its place. But this image in time suffers in the same way, so that the images alternate. The fact that a new image can suppress an old one is due to the absence of after images in the first and their presence in the second. The predominance of a bright image can be explained by the longer time required for the after image to reach such a level as shall cause suppression. The preference for an image with contours would seem to be due to the greater ease with which the after image may be removed by small deflections of gaze.

9. ERRORS OF APPRECIATION

Under this heading we include all types of abnormality in the retinal apparatus or in its central connections. The class therefore includes cases in which the image formed on the retina is in every way normal, and those in which the optical defects do not adequately explain the whole of the visual disability which experiment shows to be present. The class is found to include cases which range from slight impairment to complete blindness.

The following classification may be used :—

Group 1. Both rod and cone vision are affected, and there is thus both night blindness and total colour blindness.

Group 2. Rod vision is either affected alone, or there is slight defect in cone vision as well.

Group 3. Rod vision is unaffected, while cone vision is either altogether absent, or is found to show abnormality which affects certain colours only.

Note that any one of the above groups may be found to affect either one or both eyes, and may involve the whole or only a limited part of the retina.

COMPLETE BLINDNESS (group 1) may affect the whole of one or both eyes, or may occur in half the visual fields only. It may be limited to irregular-shaped islands or patches, or it may be found associated with central or peripheral vision. The shape of the affected area frequently gives a direct clue to the cause of the condition. The shape is best determined by means of the perimeter.

(*a*) The whole of one or both eyes is found to be blind. The disease may be congenital or may be the result of inflammation affecting the posterior parts of the eye (ophthalmoscope will confirm). Injury involving the whole of the optic nerve trunk will also cause blindness of the corresponding eye.

(*b*) The blindness involves the right or left halves of one or both retinæ only. The lesion in such cases involves the optic tracts. Tumours are the commonest cause.

(*c*) The blindness is limited to a segment of the retina when the retinal vessels are affected (*e.g.* by embolism). The ophthalmoscope will confirm.

(*d*) Blindness which affects only the periphery of the retina is due either to deficiency of blood supply (as may occur in glaucoma), defective blood (severe anæmia), or the presence of poisons in the blood.

(*e*) If blindness affects chiefly the centre of the retina, the cause is probably poisoning by either tobacco, alcohol or both. At first vision is impaired for certain colours only, but the blindness quickly becomes complete if the absorption of the poison continues.

(*f*) If irregular blind islands called *scotomata* are found in the visual fields, the cause may be inflammation of the choroid or of the retina itself, or the detachment of the retina from the choroid.

NIGHT BLINDNESS (group 2) is found in four different types of case.

(*a*) As an inherited condition. (*b*) In diseases of the liver in which bile salts are found to circulate in the blood, since these dissolve the visual purple out of the retina and therefore impair rod vision. (*c*) As a symptom of inadequate diet (lack of Vitamin A). (*d*) In disease of the retina or choroid (*e.g.* retinitis pigmentosa). In the last two cases it is usual to find colour vision affected to some extent.

The symptoms of night blindness are well described by the name. The eye does not possess the power of becoming fully dark adapted, and even a moderate degree is only attained after a prolonged stay in the dark. A photochromatic interval is not found, that is to say, when the intensity of a colour is reduced, it does not pass through an uncoloured stage (due to the rods). Purkinje's phenomenon is usually poorly developed or is not seen at all. There is no cure in the congenital cases.

COLOUR BLINDNESS. The detection of this condition is important because of the use of coloured signals in the railway and marine services. The employees of such services should be tested at frequent stated intervals, because colour blindness may develop (*e.g.* from tobacco or alcohol poisoning) in a relatively short space of time.

The methods of testing colour blindness fall roughly into three classes : (1) those of historic interest ; (2) those used by ophthalmologists for practical tests ; (3) those used for research. Holmgren's wool test is an example of the first class. It consists of a large series of coloured worsteds, the number of different shades being very great. In using the test the doctor hands to the patient selected skeins which have been found by experience to give difficulty to colour blind persons. He then instructs the patient to select from the box all the skeins that appear to him to have the same colour. His visual defect is judged by the mistakes which he makes. Since coloured

wools readily fade and get dirty in use, slips of coloured glass or beads, &c. have been used in a similar way. These tests are however no longer employed, because it is found that colour blind persons may be able to pass them without detection.

Of practical methods the lantern test would appear to yield the best results, because it gives a close imitation of a signal light as seen at different distances and under various atmospheric conditions. In using the test, the examiner

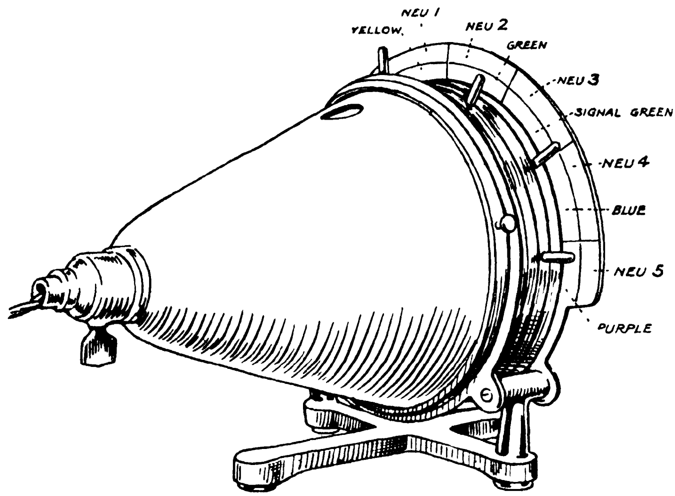

FIG. 287. The Lantern used in practice for the Detection of Colour Blindness. (EDRIDGE GREEN.)

shows the subject in turn a series of different coloured lights, and in each case asks him to state what he sees. If he makes no mistakes, the colours are shown through modifying glasses which give the effect of a signal when seen through mist, fog, rain, &c. and the answers noted. One definitely wrong answer should reject the subject, particularly if red, green or white be one of the signals misnamed. Objections have been raised to this test, because it is possible for a man ignorant of colour names 'to be failed' even if he has normal colour vision. The objection is readily met, however. When the driver of a train sees a signal, he says to himself "that is a green signal and therefore my train may proceed." But supposing all the time it were a red signal, and that he called it green through ignorance of the name, that man is as much a danger to the community as if he were in fact colour blind. No test can be too searching, and no borderline case should ever be passed; the risk is too serious.

Of tests of theoretical importance some have already been described, namely the measurement of the thresholds for light and colour, the colour-mixing apparatus and the flicker method of photometry. There is, however, another test which is found to give valuable information, namely the spectroscope test of Edridge Green. The instrument consists of a spectroscope to which is fitted two shutters, one of which may be caused to shut off the spectrum from the red end and the other from the violet. The patient commences the test by placing the shutter on the red side at the place where he sees the red begin, and this position on the wavelength scale of the shutter is noted. The patient is then told to move the other shutter until it reaches the place where red changes to orange. This wavelength also is noted. The red side shutter is now moved until it occupies the position of the violet side shutter, and the violet side shutter is now moved until a difference in colour at the two sides of the spectral area, which is thus isolated, is just not able to be seen. The wavelengths are

again noted, and the next area measured off, and so on until the violet end of the spectrum is reached. A person with normal vision will with this instrument map out between 20 and 30 distinct strips. Abnormal vision may be shown in three ways: firstly, by the ends of the spectrum being found in abnormal positions, the spectrum being shortened at the red or the violet, or both; secondly, by the isolated strips being too large and too few; and thirdly, by wrong names being applied to some of them. The value of the method is considerable because it shows the presence of all three classes of defect, those due to blindness, those caused by ignorance of colour names, and those in which the appreciation of colour is deficient.

Cases of colour blindness, when tested by the above methods, are found to show every possible variation between complete blindness and slight impairment of the colour sense. Their classification is as a rule complicated by the fact that cases are usually described in terms of one of the theories of colour vision. Most varieties of colour blindness are inherited, and are commoner in men than in women. But it may also be acquired, as explained above, in poisoning by alcohol and tobacco. Cases of colour blindness may be grouped as follows :—

(1) Cases in which the cone mechanism of the retina is not functioning. The patient is found to be colour and day blind; red is not seen at all, while the other colours are seen as different shades of grey. Vision at night is good, vision by day is complicated by the fact that the patient must not expose his eyes to a bright light, for otherwise his visual purple will become bleached and his rod apparatus therefore cease to function. Owing to the absence of rods from the fovea, this part of the retina is blind. Visual acuity is therefore low. When tested by the flicker method, his luminosity curve is found to correspond with that of twilight vision. His condition may be improved by using neutral tinted glasses fitted with a sky shade.

(2) Cases in which the cone apparatus is apparently normal, that is to say, there is no avoidance on the part of the patient of strong light, no diminished visual acuity, no foveal blindness and no inability to see red light. Yet there is absolute inability to recognise all colours, any one part of the spectrum being able to be matched by any other. Tests by means of the flicker method show a luminosity curve which corresponds to that of day vision. It would seem clear that the retinal apparatus is in every way normal; one is therefore forced to the conclusion that the defect concerns the brain centre which subserves the appreciation of colour. This view is supported by the fact that, between this extreme type and normal colour perception, there are a large number of cases which show various grades of defect. Some, for example, see two colours at the ends of the spectrum only, the intermediate portion being a neutral colour; others see three only, at red, green and blue, and so on. Since in all these cases the cones are apparently normal, they would appear to be a parallel with cases in which there is no trace of deafness, and yet there is an inability to appreciate harmony, or to tell when two notes are in tune. In both types of cases, it would seem that the higher centres of perception and appreciation are either absent or are undeveloped. As might be anticipated, therefore, instruction and practice at colour naming and colour matching benefit a certain number of these cases, so that it is sometimes found that after such instruction the less abnormal cases are not readily detected. If, however, they are tested in a poor light, they are found to make mistakes which a person with normal vision would not commit. As these are the circumstances under which signals have frequently to be recognised, it is for this reason that the lantern test with its modifying glasses is so valuable.

(3) Cases in which certain parts of the spectrum are not seen at all. This condition frequently affects the red end of the spectrum, but it may be found

in other parts. The principal effects to be noted are: (i) an abnormal type of luminosity curve, as examined by means of the flicker method; (ii) the requirement of different amounts of the primary colours in order to match a given spectral colour, as compared with individuals of normal vision; (iii) inability to recognise the normal number of different hues in the part of the spectrum affected, as shown by the spectroscope test; (iv) shortening of the spectrum, if either of the ends of the spectrum is affected. The condition would appear to be directly traceable to abnormality in the colour receiving apparatus of which the cones form an important part. These cases therefore show quite distinct features, which at once differentiate them from those of class 2. A typical example of a case belonging to class 3 will now be described, namely that in which there is shortening at the red end of the spectrum. The flicker test shows that the luminosity curve for different parts of the spectrum has its maximum in the green, instead of in the yellow. Further, the curve does not extend so far into the red, or show such high values in the orange as the normal curve. This curve therefore explains the apparent shortening of the spectrum. Colour mixture experiments show that the patient can match mixtures of green and blue with white light. When required to match yellow, he uses an excessive amount of red, and correspondingly less green than the normal sighted; this test again shows the deficiency of the red sensation. Tested by the spectroscope, it is found that, besides the shortening at the red end of the spectrum, there is also an inability to distinguish the normal number of hues at the green and yellow. This effect is readily explained, because the difference between green and yellow shades largely depends on the varying extent to which they stimulate the red sensation. When the red sensation is absent, it is clear that the differentiation of greens and yellows must suffer.

Effect of Intensity on Colour Vision. It is well known that there is a certain range of intensity over which the appreciation of colour is at a maximum, and that at high and low intensities appreciation is diminished. Thus at low intensity the spectrum will appear shortened at both red and violet ends, and with the spectroscope test perhaps 10 monochromatic areas will be mapped out instead of the normal 20 to 30. At high intensity, on the other hand, the spectrum appears to extend further than usual at both red and violet ends, but again it is found that the number of apparently monochromatic areas is considerably reduced. In the one case it would seem that the impulses received by the brain from the cone mechanism are so feeble that appreciation is diminished, and in the second that a powerful colour stimulus arouses all thrèe sensations indifferently and therefore makes the differentiation of colour difficult.

Peripheral Vision. Experiments with the perimeter show that there is under ordinary circumstances a reduced appreciation of colour in the periphery of the retina. Also in an annular zone round the macular region there is red-green blindness but full appreciation of yellow and blue. Outside this area coloured objects are seen in different shades of grey. More careful experiments show firstly, that there is no hard and fast line limiting the zones, but a gradual diminution of colour perception on passing in any direction from the centre to the periphery; and secondly, that intensity plays a most important part, an increase being sufficient to effect normal colour perception, even in quite peripheral vision. The red-green blindness found at one intensity might be due either to an absence, or more probably to a weakness or deficiency, of either the red or the green sensations, or to defective appreciation on the part of the higher centres in the brain. In the first

case there would be an abnormal shape to the luminosity curve, such as is found in fact in red or green blindness, whereas in the second case the luminosity curve would be similar to that of normal vision. Experiments are said to show that the curve is normal, and therefore the cones in the periphery must be in every way normal, a supposition which is borne out by the correct appreciation of colour at high intensity. Why then, it may be asked, is colour vision reduced at the periphery if the cones are normal? The answer is, probably, firstly, that the number of cones is greatly diminished, and secondly, that the effective area of the pupil is much less at the periphery than it is at the centre of the retina. We have seen in Section I that the threshold necessary for the appreciation of colour depends on the size of the area of the retina which is receiving stimulation. The larger the area the lower can the intensity be. Therefore one unit of intensity falling on 100 cones is equivalent to 100 units falling on one cone. Now consider the relative conditions at the fovea and the periphery; at the fovea let us suppose there to be 100 units of intensity falling on 100 cones, then at the periphery there will be but 50 units (because the effective area of the pupil is less owing to the rays entering obliquely), and these will fall on perhaps two cones only. Whereas in the first case there are 10,000 cone units, in the second there are 100 cone units only, and it is therefore to be expected that appreciation of colour would be decreased, in the same way as it is at the fovea under reduced illumination.

Ferree and Rand projected the spectral colours in turn on the periphery of the retina in such a way that the intensity of illumination for them all was equal to the intensity at the fovea. They found that red and blue light could be seen as far into the periphery as white light, but that green could not.

10. THEORIES OF COLOUR VISION

The value of a theory to science is as much due to the fresh lines of research which it indicates, as to the explanation which it offers of the already ascertained facts. The theories of vision, therefore, are of value in spite of the fact that they do not at the present time offer a complete account of the retina and its functions.

THE DUPLEX THEORY of von Kries states that the retina shows two different types of response, one for low illumination and one for high, *i.e.* twilight vision, which is colour blind, and day vision, which responds to colour. The view that the rods with the visual purple supply the former, whereas the cones provide the latter, is one widely held and is already familiar, because it has been made the basis of the description given in previous sections. The evidence on which this opinion is based may with advantage be repeated because of its importance.

Twilight vision is found in those parts of the retina where there are rods; it is not present, therefore, at the fovea centralis, which consists entirely of cones. If a spectrum be examined, it is found that the colour with the greatest luminosity is the green, but that red rays are not seen at all. The form of the luminosity curve is identical with the bleaching curve of visual purple, and this pigment occurs only where there are rods. The acuity of twilight vision is low, and is explained by the fact that many rods as a rule send their impulses through one and the same nerve fibre.

Day vision is most highly developed in the fovea, from which rods are absent. Not only are the cones at the fovea placed very closely together, but it would appear that each cone connects to one nerve fibre only; in this way the high visual acuity is explained.

It has been suggested recently that the following modifications should be made in the duplicity theory of von Kries:—

1. That the cones do, in certain cases, function to some extent in night vision, thus retaining one of the primitive characteristics of rods, from which on morphological grounds they appear to have been developed.
2. That the fovea contains some visual purple, this being necessary in order that

the cones may function in night vision as above, or possibly for the green sensation of vision.

3. That the rods play some part in day vision, adding their response to that of the day cones.

These modifications of the duplicity theory concern detail more than they do the basis of the theory, and do not appear to detract at all from the strength of its position. Hence, so far as the relative rôles of the rods and cones are concerned, there would not appear to be any room for speculation. Such is not the case, however, with regard to colour vision because, of the various hypotheses that have been so far advanced, none have been found to offer a feasible explanation of all the known facts, or to leave no other possible alternative. A brief account of the rival theories may with advantage be given.

YOUNG'S HYPOTHESIS states that there are in the retina three different types of cone, each being so made as to respond to one of the three fundamental colours, namely red, green and violet. The impulses from these cones are so combined in the brain that they give a complete picture of the separate coloured images. When all three types of cone are equally stimulated, a colourless sensation results. Each visual unit may therefore be regarded as consisting of three cones, one of which responds to each of the fundamental colours. From this we should expect that the limit to the acuteness of vision would be reached when the separation of the images on the retina is not less than the diameter of such a unit. But the diameter of the foveal cones is approximately 0 0025 mm., and therefore that of a unit would be roughly 0 004 mm. Now it is found by experiment that the limit to the acuteness of vision is reached when the retinal images are separated by about 0 002. It is therefore clear that the unit cannot be larger than one cone, and that as a result each cone must be capable of responding to all three fundamental colours. In consequence of this Helmholtz made the suggestion that there are three different chemical substances, each of which undergoes alteration under the influence of one of the three fundamental colours. The breakdown products thus formed stimulate the cones in proportion to the amount in which they are present, the cones in this respect being comparable to the taste buds of the tongue. In this way each cone can respond to all three colours, and also to white light, and therefore the requirements of visual acuity are satisfied. As to what these chemical substances might be, we know nothing; it has been suggested that the substance responsible for the perception of blue is a pigment discovered by Kuhne, called visual yellow, and visual purple might from its absorption curve provide the pigment for the green, but at present we have no evidence for this. It should be noted that the three sensations are brighter and more saturated than the three fundamental colours with which they may be said to correspond. This follows from the researches of Maxwell and Abney, which showed that each of the fundamental colours stimulates to some extent the other sensations beside its own. Thus blue light stimulates the green and red sensations to a certain extent, green similarly the red and blue sensations, but red the green slightly, and the blue not at all. This view as to the greater saturation of the sensations finds some confirmatory evidence from the increase which the saturation of a colour undergoes after the eye has been stimulated by its complementary colour.

Having thus briefly outlined the hypothesis of Young and Helmholtz, its relationship with the results of experiment may receive consideration.

1. The results of colour mixture are all adequately explained. Since to each of the fundamental colours there is a corresponding sensation, and since mixtures of the fundamentals can produce the whole range of colour, it follows that corresponding stimulation of the sensations and their resynthesis in the brain fits in with the facts.

2. The various classes of colour blindness which show abnormal types of luminosity curve, abnormal colour mixture values, and possibly also a shortening of the spectrum, are readily explained by supposing one of the sensations to be defective or absent. For example, cases which show a shortening of the red end of the spectrum are stated by the theory to have a deficient red sensation. The luminosity curve calculated on this basis is found to fit closely the curve found by experiment in these cases of colour blindness. The hypothesis would appear, therefore, to be able fully to explain the various cases which fall in this class.

Certain objections have, however, been advanced which it would be well to examine.

(*a*) That it does not explain why the red blind and the green blind state that the ends of the spectrum, as they see them, are yellow and blue, whereas it would be expected that they would say green and blue if red blind, and red and blue if green blind. An explanation of this behaviour can be readily obtained by examining the forms of the red and green sensation curves, Fig. 288, for in both red and green blindness a yellow light is that which stimulates the remaining sensation most strongly, without at the same

time involving the blue. In both types of case, therefore, both red and green are regarded as being but degraded yellows, and the spectrum is therefore named accordingly. (*b*) That the hypothesis does not explain why these same cases call white white, instead of bluish-green or purple. This is explained by the fact that a colour blind person will call white what his fellows who have normal colour vision call

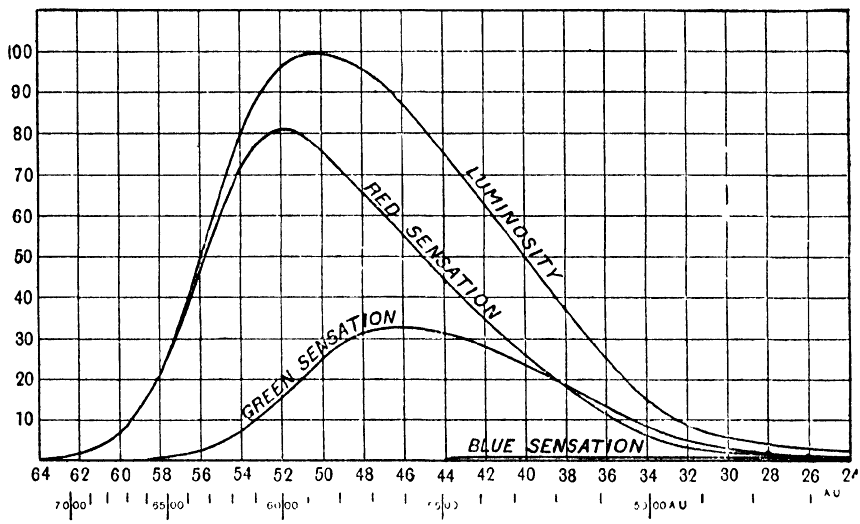

FIG. 288. The red, green and blue Sensation Curves and the Luminosity Curve of white Light. Luminosity vertical, wavelengths horizontal. (ABNEY.)

white, because he has learned his colour names from them. In the same way a green blind person will not call the leaves of a tree by a wrong colour, although he will readily err if a piece of paper of the same colour as a leaf be handed to him.

The various types of colour blindness which have normal luminosity curves cannot be explained by the hypothesis without some further elaboration. As indicated, however, they fit in well with the supposition that it is not the eye, but the higher centres which are at fault. The impulses which travel up the optic nerve are in every way normal; the error occurs in their interpretation. This would appear to be a reasonable explanation which agrees with the other postulates of the hypothesis. It has been advanced by Edridge Green as part of another hypothesis of colour vision, which will be given later.

3. Contrast, after-images and allied phenomena are not adequately dealt with. Helmholtz regarded contrast as an error of judgment, but Hering showed conclusively that such could not be the case. McDougall's hypothesis, which is to a large extent based on that of Young, will be found to add the features that are required for the explanation of after images and contrast. This theory has received valuable confirmation at the hands of Allen. An account of his experiments will be found on p. 450.

OTHER HYPOTHESES OF COLOUR VISION

In addition to Young's theory, numerous other theories of colour vision have been advanced, many of which are of historic interest only. Brief reference will be made to three of these.

HERING'S HYPOTHESIS states that there are in the retina three substances which are always tending to dissociate into their components. They are, however, either replaced or built up again from substances in the blood as quickly as they are destroyed. There is thus equilibrium between anabolism and catabolism when the eye is unstimulated, and while this is the case no nerve impulses travel to the brain. Now each of these substances is dissociated by one of the following colours, red, yellow, white; and is built up when green, blue or black fall on the retina. Thus one substance will break up when red light falls on it, and will recombine when green does. There is thus a red-green, a yellow-blue, and a white-black substance. When a coloured

light falls on the retina these three substances are broken down, or are built up, in varying amounts and corresponding impressions sent to the brain.

EDRIDGE GREEN'S HYPOTHESIS states that the function of the rods is to secrete visual purple. This pigment, under the action of light, stimulates the ends of the cones, and causes them to send impulses to the brain which vary according to the wave-length of the light and its intensity. The rods are, on this view, merely secretory organs, and take no other part in vision. The impulses, having reached the brain, go first to a light perceiving centre, and then to another especially developed for the appreciation of colour. In this colour centre there are three separate mechanisms, which correspond roughly with the red, green and blue fundamental colours, but which may respond to other frequencies than those to which they approximately correspond. Suppose, for example, that a monochromatic yellow light is falling on the retina; it is absorbed by the visual purple and thus stimulates the ends of the cones. These then send up the optic nerve impulses which have a mean frequency corresponding to yellow light. These impulses, having reached the colour centre, stimulate the red and green mechanisms respectively, in proportion to the energy which each receives.

MCDOUGALL'S HYPOTHESIS is not antagonistic to Young's theory, as the two previous views have been, but adds valuable suggestions as to the causation of contrast and after-image phenomena, points to which the original theory gave little or no attention. McDougall also accepts the duplicity theory. He commences by stating that there are four centres for the two eyes, namely red, green, blue and white (the mechanism of which is the rods), and that these centres are distinct and have no anatomical identity. Between these centres there is antagonism, the red centre of one eye against the green and blue centres of the other and also to a less extent against those of itself. In this way one can explain not only binocular, but also monocular rivalry. Contrast is interpreted in a somewhat similar manner; thus, if the object looked at consists of a red area on a grey field, the red stimulus inhibits the appreciation of red in the surrounding field, and therefore causes it to have a blue-green colour, a deduction which is confirmed by experiment. After-images are dealt with in a somewhat similar manner.

With the evidence that has accumulated up to the present before us, there appears to be more in favour of Young's hypothesis than is to be found for its rivals. Further than that, it is not at present advisable to go.

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11. BINOCULAR VISION

Binocular vision may be defined as the co-ordinated employment of two separate visual organs in order to produce a single mental impression. The advantages of binocular as opposed to monocular vision are :—

(1) Optical defects of one eye are less important, since they are masked by the well-defined images of the other eye.

(2) Defective vision in parts of the visual fields of both eyes is hidden, so long as the defects do not affect the same parts of both fields. Thus under ordinary circumstances, each blind spot does not obtrude itself because in the other eye the image of the same part of the field falls on functional retina.

(3) The combined fields of the two eyes are larger than either alone because, while the features restrict the nasal halves of the fields of both eyes, the combined field contains the unrestricted temporal areas of both retinæ.

(4) Binocular vision under certain circumstances provides a very accurate perception of depth, size and distance, which is called stereoscopic vision.

In order that there should be binocular vision the following conditions should be complied with :—

(1) The fields of the two eyes must overlap. Animals in which the eye axes are parallel have the greatest overlap, and therefore possess the most perfect binocular vision.

(2) Approximately similar images must be formed on the retinæ, because

if this condition is not satisfied, antagonism between the images will occur, as described above, and first one image and then the other will be presented to consciousness.

(3) The retinæ must possess physiologically corresponding points, in order that similar images formed on them may produce one conscious impression.

(4) The external eye muscles must so adjust the visual axes, that the centres of the fields of the two eyes coincide with the images of one and the same object. This adjustment is called fixation. It is sometimes described as the intersection of the visual axes at the point fixated.

(5) The oblique muscles must rotate the eyes about their axes, until corresponding retinal points occupy corresponding meridians.

The rotation adjustment is necessary because otherwise identical points of the retinæ might not correspond, even when the centres did, so that one image would appear tilted at an angle with the other.

FIXATION is partly a voluntary act and partly a reflex process. The former is shown by the fact that the eyes may be directed towards an imaginary object a short distance from the face, so that the eye axes are strongly converging and the accommodation correctly adjusted to the same plane. The presence of a reflex phase is well shown by the fact that no effort of the will is required to sustain fixation on an object in which we are interested, and also by those cases in which when once an object has been fixated, there is found to be considerable mental difficulty in turning the gaze elsewhere. Rotation fixation, on the other hand, appears to be entirely reflex. In order that fixation should be obtained when the gaze is directed in different directions, it is necessary that there should be close association between the synergic muscles of the two eyes. This is at all events assisted by the anatomical arrangement of the 3rd, 4th and 6th cranial nerve nuclei which has been described previously (see page 390). Not only are the corresponding nuclei on the two sides connected by transverse fibres, so that either the superior recti or the inferior recti move together, but the external rectus nucleus of one side is joined to the internal rectus nucleus of the other by the dorsal longitudinal bundle, so that the eyes deviate together to right and left. Similar connections are to be found between the nuclei of the superior and inferior obliques. The relations of these nuclei to the cerebral cortex have been ascertained by electrical stimulation. It has been found that stimulation of the median third of the limb of the angular gyrus on either side causes both eyes to be turned to the opposite side. The right gyrus therefore connects with the nuclei of the right internal rectus (3rd) and the left external rectus (6th). Since both these nuclei are on the left, the fibres from the gyri must cross in order to reach their corresponding nuclei: this they do at the level of the superior corpora quadrigemina. The angular gyri are connected to both the frontal and occipital parts of the cortex, so that voluntary movements of the eyes, and also movements under the action of light, can be carried out. Experimental stimulation of the semicircular canals is found to cause conjugate deviations of the eyes. But stimulation of the canals is effected naturally by a rotation of the head, as has been described. The conjugate deviation of the eyes thus initiated causes the gaze to remain stationary on external objects in spite of the head movements.

THE FIXATION REFLEX. The way in which involuntary fixation is brought about may be described as follows :—When an image falls on the periphery of the retina, an impulse reaches the oculo-motor nuclei in the manner described above. Thus, suppose the image to come from the right,

it will fall initially on the left halves of both retinæ, and impulses will therefore travel to the left calcarine cortex. From here, they will pass to the right-hand centres, causing impulses to travel to the left internal and the right external recti. Both eyes are therefore directed to the right, the movement being such as to bring the image on to the fovea. But as the fovea is approached the impression sent to consciousness becomes increasingly distinct, owing to the higher acuity of the fovea. If the fovea is passed, the image begins to become indistinct again, and therefore the movement of the eyes is checked as soon as the image has reached the fovea. (See also page 425.) If the acuity of the fovea is reduced by disease or by working in a bad light, the definition of the image does not sharply improve as the fovea is reached, and therefore the movement of the eyes is not checked until the image has reached the periphery again. But here the degradation of the image calls for the reverse process, which again causes the image to pass over the fovea. Repeated oscillations of the eyes therefore occur, which are called *nystagmus*. The condition is met with in the day-blind, since cone vision is defective in persons whose visual acuity has been lowered by working in a dull light, *e g.* miners, and also in cases of poisoning by tobacco or alcohol.

THE HOROPTER. Theory shows that, even when fixation is properly effected, so that corresponding retinal points occupy the same meridians, images formed on the retinæ do not necessarily fall on corresponding points. For this to be the case, it is necessary also that the objects from which those images are formed should occupy certain definite positions in relationship with one another. For example, if an object 10 feet from the eye is fixated, the images of other objects on either side will only fall on corresponding points if they lie on a circle of 5 feet radius, the centre of which is between the observer and the object fixated. For calculation shows that only then are the images formed on the two retinæ at the same distances from the centres. The form of the curve, which is called the horopter, is found to change with the different directions of the gaze. When the gaze is directed to a point on the floor, it is stated that the horopter almost corresponds with the plane of the floor.

MONOCULAR DEPTH PERCEPTION. The perception of depth with the single eye is found to depend on a number of different factors, which as a rule operate together :—

(1) The apparent size of objects, the dimensions of which are known. Thus the size of a man being approximately known, his distance away is deduced from the size of the image which is formed on the retina. The further away he is, the smaller his image will appear.

(2) The colour of an object being known, the effect of distance in modifying that colour is used in depth perception. Thus trees, which when near look yellow-green, when seen at a distance through an intervening layer of haze appear blue-green or even blue. This fact is made use of by artists for expressing distance ("ærial perspective.")

(3) The partial obstruction of a distant plane by objects nearer to the observer.

(4) The shadows which one plane casts upon another.

(5) The intensity of the light which is reflected by the object frequently varies with its shape and position. For example, the shape of a solid sphere can be accurately inferred from the distribution of intensity over its face.

(6) By linear perspective, which may be defined as the geometrical arrangement of lines in the image formed on the retina. Thus the lines of a tennis court seen diagonally from one side are all found to converge to one or other of two points on the horizon.

(7) By the intersection of objects with the horizontal plane. Thus the

positions of trees in a field may be inferred with some accuracy, if the positions of the roots of the trees in relationship with the boundaries of the field be observed.

(8) By parallax, that is, the apparent movement of objects in relationship with one another. Thus if a middle plane be looked at, and the observer

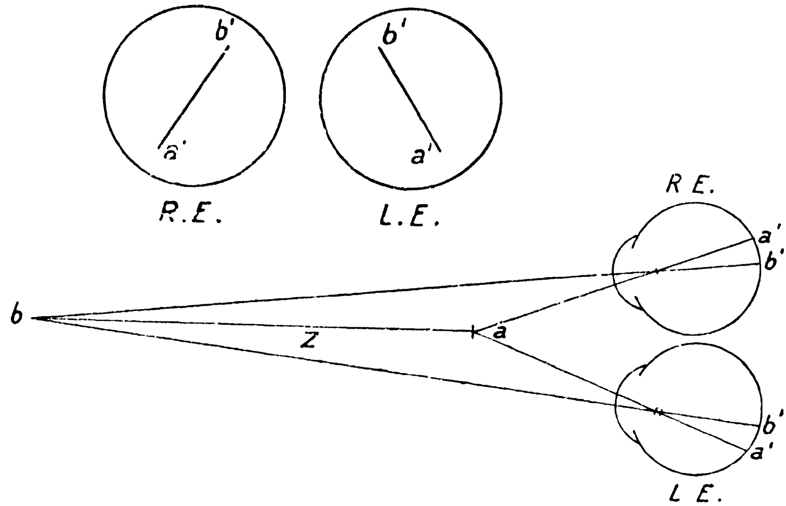

FIG. 289. The eyes are directed to the point *b*. A thread hung obliquely at *a* gives rise under these circumstances to the images shown in the upper figures —*i.e.* two images which do not lie on corresponding points. Nevertheless the thread is seen as single.

moves, it will be noticed that objects in a plane behind appear to move in the same direction as the observer, while those in a plane in front appear to move the opposite way. Even when we are standing still, we are all the time making involuntary movements which cause the development of parallax. This process is probably one of the most important in producing the monocular effect of depth.

(9) By the effort of accommodation required to focus an object sharply. In man the accommodation is found by experiment to give little or no perception of depth, possibly because the function is involuntary. It is thought that in birds, in which the ciliary muscles are striated and are under voluntary control, the accommodation may give valuable information of distance.

All the above factors operate together to produce an appreciation of distance which as a result of experience reaches a very high order, and with the exception of the last two, are used by the artist to produce the effect of solidity and reality. Any good picture shows us that the result can be very convincing.

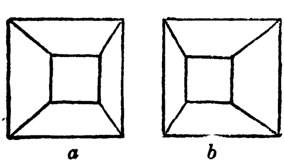

FIG. 290. To show the difference in the Images of a Truncated Pyramid as given by the right and left eyes.

STEREOSCOPIC VISION is the binocular perception of depth. It consists of all the factors which operate in the case of each eye separately, and in addition uses :—

(1) The convergence of the eye axes which is necessary in order to cause images of near objects to form on the fovea simultaneously.

(2) The dissimilarity between the images which are formed on the two retinæ. (Fig. 290.)

That convergence has very little effect on the perception of distance can be proved by placing weak prisms, either base in or base out, in front of the eyes and in this way changing the convergence of the eye axes without changing any other condition. It is found that the apparent positions of objects are unaffected.

That there is dissimilarity between the images formed on the retina can easily be proved by experiment. Thus, if the gaze be directed towards a distant point, and the finger be held a short distance from the nose, the finger appears to be to the right of the distant point with the left eye and to the right with the left. If two photographs be taken of the same scene, but with the camera for the second photograph three inches to one side of its position for the first, it is found that, when the two negatives are placed so that objects on the horizon correspond, there is a lateral difference of position in the case of all other objects situated nearer to the camera. Measurement shows that the nearer the object the greater the difference in position. Since this is the case, it is clear that only images in one plane can be formed on corresponding retinal points; images in all other planes must fall on points which are discrepant. Two questions therefore arise: (1) Do we see such objects doubled? (2) If we see a single image only, is it because one of the images is displaced from consciousness by the antagonism of the other? An answer is given by the following experiment:—A Brewster's stereoscope is taken, the optical arrangement for which is shown in Fig. 291. At B and B′ two similar lantern slides are placed which show a view of any distant objects. On looking through the instrument towards the point S, the directions of the rays are changed so that the images of the slides are seen to overlap one another. By shifting one of the slides the images may be made to fall on corresponding points of the retinæ, and they then form a single combined picture. In front of these slides is now placed another pair of slides which show the photograph of an index mark. If the indices are adjusted so that they occupy corresponding positions in relationship with the objects on the slides below them, on looking into the instrument it will be seen that these marks appear to lie in the same plane as the distant objects placed on the slides below them. If one of the index marks be moved towards the axis of the instrument, it will be seen on looking into the eyepieces that the indices now appear to lie in a plane considerably in front of their previous position, in fact that the closer they are placed together, the nearer do they appear to the observer. But the indices do not show double images unless they are moved a considerable distance together, and then the effect of distance ceases. If one of the index slides be removed and the other be moved towards and away from the axis of the instrument, the index is not found to shift its plane towards or away from the instrument. This shows that for position to be appreciated both images must be presented to consciousness simultaneously without appearing double.

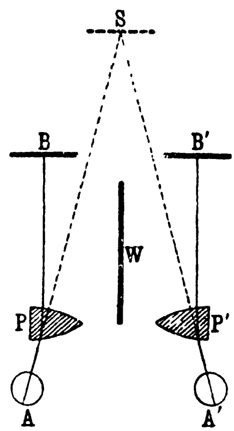

FIG. 291. Brewster's Stereoscope.

THE ACUITY OF STEREOSCOPIC VISION has been investigated in such a way that other factors which normally assist distance perception were excluded. Two methods

have been used: (1) to adjust the position of a thread which lies between and parallel with two other threads until they all appear at the same distance from the observer; (2) to observe the fall of small coloured bodies of unknown size, and then to state the position of the line of fall in relationship with a fixation mark. The former method at 2 metres distance shows an average error of 1·5 mm., the latter method at the same distance an error of 40 mm. The difference between the results of the two methods is considerable; but it should be noted that in the fall method the object is seen only for 0 02 sec. If in the thread method the threads be placed horizontal it is found that the appreciation of distance is greatly impaired. The greatest acuity is found when the threads are vertical. If, however, the head is turned so that the line joining the two eyes is vertical, the greatest acuity is found when the threads are horizontal. This would be expected if the appreciation of distance is greatest when the parallax of the objects at the two eyes is greatest. Experiment shows that the recognition of position in relationship with a definite fixation mark is much more accurate than recognition of absolute distance in which there is no point of reference. Thus it is well known how inaccurate the estimation of the distance of a single source of light at night may be.

HYPOTHESES OF DEPTH PERCEPTION. Javal's view was that the movements of the eye muscles, which are necessary in order to direct the gaze from objects in one plane to those in the next, caused impulses to travel to the brain which are interpreted in terms of distance. This view was ruled out by the fact that images which are formed on the retina for a short length of time only (0·02 sec.) can be perceived in relief.

HERING'S HYPOTHESIS was that it is the formation of similar images on points of the retinæ that do not correspond which causes distance perception. If the disparation is crossed, the object appears nearer than the fixation mark by an amount which depends on the amount of the disparation. If, on the other hand, the disparation is uncrossed, the object is recognised as being further away. Hering supposed moreover that crossed disparation acts as a stimulus to convergence and accommodation, while uncrossed produces the reverse effect. We may now inquire how this hypothesis fits in with the facts. To commence with, if depth depends on disparation, it is clear that, when we perceive objects lying in different planes, we must subconsciously group them according as they fall on corresponding retinal points, or on points which are discrepant by one, two, three or more cone widths, and whether the discrepancy is crossed or uncrossed. The amount of the discrepancy must be some whole number of cone widths, because it is clearly impossible to stimulate half a cone with one impression and the other half of the same cone with a different one and obtain two distinct sensations. It is clear that space must be divided, so far as stereoscopic vision is concerned into a number of concentric shells, the centres of which correspond with the position of the observer. Now the thickness of these shells can be readily calculated · at 1 metre they are found to be 2 mm. thick, at 10 metres 200 mm. thick, and at 100 metres 17 metres thick. If we are looking at a fixation mark 10 metres away, objects 100 mm. nearer to and 100 mm. further from the observer will lie in the thickness of one and the same shell, and will therefore appear the same distance from the observer. Objects between 100 and 300 mm. nearer to the observer will lie in the shell corresponding to one cone discrepancy, and will therefore be appreciated as being at a different distance from the observer, appearing nearer if crossed, and further if uncrossed. The same reasoning applies to objects at other distances. If this calculation is correct, it should be necessary to place objects more than 100 mm. from a fixation mark, which is itself placed at 10 metres, in order that a difference in the distance from the observer should be appreciated. Greeff found by experiment that $\frac{1}{50}$th the distance of the fixation mark was necessary (*i.e.* 200 mm.), the observations being instantaneous ones. If time be allowed for prolonged observation, greater accuracy in the appreciation of distance is obtainable, because different points of fixation can be used. Suppose for example that two objects 20 mm. apart be examined at a distance of 10 metres, under instantaneous observation they will appear identical as described above; but if the examination be made more carefully, it will be found that, on fixating a point a mean distance of 100 mm. away from the objects, the distance between the two is suddenly appreciated because the demarcation between two shells now falls between them. It is in this way that the accuracy of extended observation becomes greater than that obtainable with instantaneous. The limit reached by experiment is stated to correspond to a displacement at the retina corresponding to $\frac{1}{10}$th the diameter of a cone. The corresponding values for the acuity of stereoscopic vision would be $\frac{1}{10}$th those given above, namely 0·2 mm. at 1 metre, 20 mm. at 10 metres, and 1·7 metres at 100 metres. Hering's view would therefore appear to agree well with the results of experiment. It remains to consider the type of cortical mechanism that would be necessary for the estimation of the discrepancy between the images. One type may be briefly described

as follows :—To a number of parallel "planes" in the left side of the cortex are connected the terminal ends of the nerve fibres from the left halves of the two retinæ. At the middle plane fibres from exactly corresponding retinal points are connected together. At planes which lie superficially to the middle plane are connected other terminations from the same fibres but with a crossed lateral discrepancy of one cone in the 1st plane, two cones in the 2nd plane, three cones in the 3rd plane, &c. At planes which lie deep to the middle one other terminations from the same fibres are connected, but with an uncrossed lateral discrepancy of one, two, three, &c. cones as the case may be. On looking at a fixation mark on a uniform background, therefore, a series of impressions of the mark will be formed on all these planes, but in the central one only will they exactly agree, for in all the others the lateral discrepancy will cause the impressions to be duplicated. In all these other planes there will thus be antagonism, first one image and then the other predominating. When these images are combined in consciousness, the stable image from the central plane will suppress the unstable ones from all the other planes, the result being a single picture of the fixation mark. If there are in front of the fixation mark other objects lying in planes at different distances from the observer, the impulses sent up by the cones to the cortical plane will not correspond at the central plane, because their images no longer fall on corresponding points, but they will correspond in the superficial planes where the discrepancy of their images agrees with the discrepancy of the nerve connections. These other planes will therefore predominate according as each contains the identical images, and when they are combined in consciousness these planes will suppress all the others. Since each cortical plane represents a certain lateral discrepancy, it must also represent a certain distance from the fixation mark. If consciousness recognises the plane in which a stable image is formed, it also must appreciate the distance of the object lying in that plane from the fixation mark. This would not seem any more difficult than the localisation of a touch on the skin. So far as we are able to judge there is nothing inherently impossible in the arrangement of the hypothetical cortical mechanism which has been outlined above, and therefore Hering's theory would appear to be very plausible.

CHAPTER XXVI

HEARING AND SPEECH

1. THE ORGAN OF HEARING

Sound is conducted in the form of a wave motion. When sound is conducted through the air each sound wave consists of two phases; during one phase the pressure in the air is above the atmospheric pressure, during

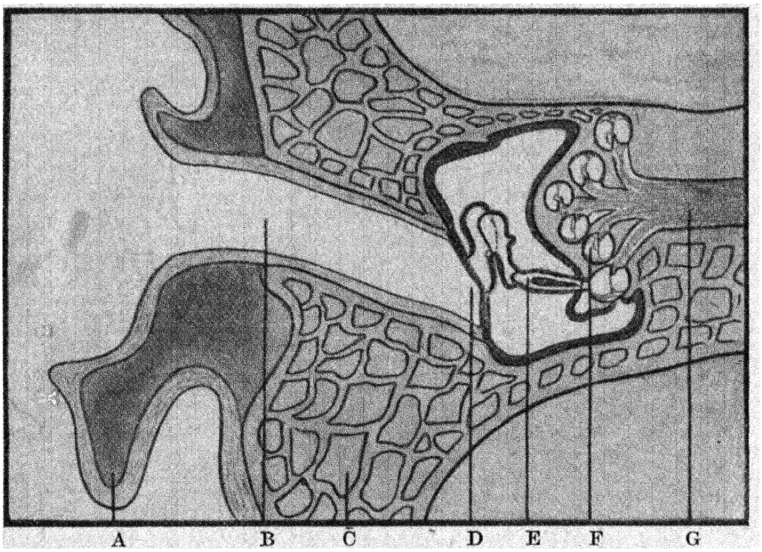

FIG. 292. Diagrammatic View of Auditory Organ.

A = Lobule of Pinna.
B = External Auditory Meatus.
C = Mastoid Air Cells.
D = Drum Skin
E = Ossicles.
F = Cochlea.
G = Acoustic (Auditory) Nerve.

the other phase it is below the atmospheric pressure. These alternate variations of pressure are conveyed to the drum skin of the middle ear *viâ* the external auditory meatus when sound waves reach an observer.

RECTIFICATION BY MEMBRANA TYMPANI. It should be noted that the drum skin has a different mode of attachment to that provided for the cone of a moving coil loud speaker. The latter is so flexibly mounted that it can move with equal facility both inwards and outwards, and so can perform a sine wave motion. The pull of the tensor tympani muscle, however, stretches the attachments of the drum skin to the meatus, so that it is only possible for the drum skin to move inwards if there is stretching of these attachments, whereas it is possible for it to move outwards by stretching the tensor tympani muscle. Of these two movements, the former is much more difficult than the latter, and thus the movements of the drum skin tend to be asymmetrical about the position of rest, so that a sine

wave motion applied to it would become distorted. In consequence of this asymmetrical movement of the drum skin, which is called "rectification" by physicists, certain tones not originally conducted by the air to the ears may be generated in the ears themselves and are heard as if they were conducted to the ears by the air. When a weak tone is being heard (below an intensity level of about 45 decibels) the movements of the drum skin and ossicles are linear, that is, the variations of pressure in the air cause corresponding movements according to Hooke's law. Under these conditions no ear-produced tones are heard. When a stronger tone is being heard the movements of the drum skin and ossicles become asymmetrical since Hooke's law is obeyed for an outward movement from rest, but is disobeyed for an inward movement from rest. Ear-produced tones are now heard consisting of even numbered harmonics. When a still stronger tone is being heard, such that Hooke's law is disobeyed both for movements inwards and for movements outwards, then odd numbered harmonics appear in addition to the even numbered ones.

When two pure tones are simultaneously received by an ear, then, in addition to the even harmonics for both tones and (if the tones are loud enough) the odd harmonics of those tones, it is found that there are also present both summation tones and difference tones. Suppose the two pure tones to be 700 and 1,200 cycles per second, then the following summation tones can usually be identified $(700 + 1{,}200 =)$ 1900; $(700 \times 2 + 1{,}200 =)$ 2,600; $(1{,}200 \times 2 + 700 =)$ 3,100; $(700 \times 2 + 1{,}200 \times 2 =)$ 3,800; $(700 \times 3 + 1{,}200 =)$ 3,300, and sometimes others as well. The following difference tones are also usually present $(1{,}200 - 700 =)$ 500; $(1{,}200 - 700 \times 2 =)$ 200; $(1{,}200 \times 2 - 700 =)$ 1,700; $(1{,}200 \times 2 - 700 \times 2 =)$ 1,000; and others generated by the ear in a similar manner. Consequently the brain receives a somewhat distorted sound picture owing to this rectification by the drum skin. This subject will be found referred to in greater detail on p. 479 under "combination tones."

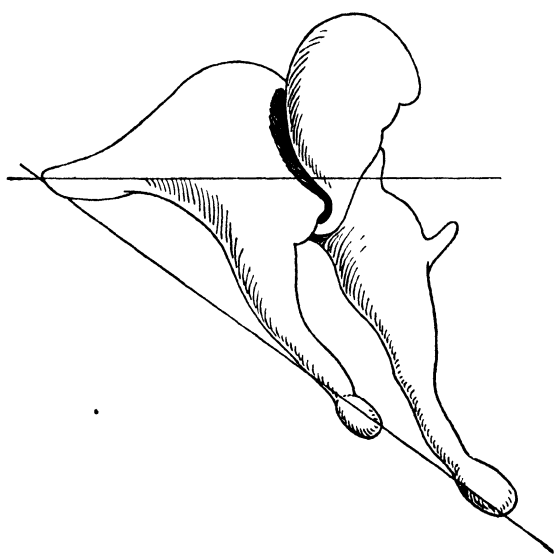

FIG. 293. To show the Relations of the Malleus and Incus to one another.

The shaded area between the two bones shows the articular surfaces which connect them. The overlapping of the two bones at the lower part of these surfaces is well shown. It is this arrangement which causes motion to be conveyed from one to the other.

THE OSSICLES. Stretching across the tympanum, from the membrana tympani to the outer wall of the internal ear, is a chain of ossicles, which are named respectively the malleus, the incus, and the stapes (Fig. 293).

THE MUSCLES found in the tympanum are the tensor tympani, which is attached to the handle of the malleus, and the stapedius attached to the base of the stapes. The tensor is innervated by a motor branch of the 5th cranial nerve, and when it is

stimulated it draws the handle of the malleus inwards and so increases the tension of the tympanic membrane. At the same time the plunger of the stapes is displaced into the oval window, thus putting compression on the contents of the internal ear. The contraction of the tensor has been supposed to have a protective function and has been compared to the sphincter pupillæ (Helmholtz). Others hold that it modifies the response to low and medium tones, but even here there is a divergence of opinion because, while some hold (probably correctly) that the tensor by its contraction decreases the natural period of the drum and thus enables it to respond to rapid changes of phase and high tones, others have held the opposite view. Observation shows that contraction occurs when sounds (particularly tones of high pitch) fall on the drum, and that the contraction is bilateral, even if the stimulus be only unilateral. The reflex therefore travels *viâ* the auditory nerve to the motor centre of the 5th nerve.

Since the tensor tympani is uncontracted when no sounds are falling on the ear, it allows the drum to go slack and therefore tends to prevent this membrane from becoming stretched through being continually in tension.

The stapedius muscle is innervated by a twig from the facial nerve. Its function is problematical. Some say it antagonises the tensor by decreasing the tension on the drum; others that it reduces the tension on the contents of the internal ear by diminishing the pressure of the stapes on the oval window. Hartridge's view is that the function of this muscle is to cause the body of the incus to engage with the spur of the malleus with sufficient force to prevent chattering and lost motion when the vibrations are being transmitted from one bone to the other.

THE EUSTACHIAN CANAL is a tube about 35 mm. in length, and connects the middle ear with the pharynx. Normally it is kept closed and hence the respiratory rhythm does not affect the pressure in the tympanum, and the noise set up by the flow of air and by the voice not heard. When the canal is shut, the middle ear becomes a closed chamber, which appears to increase the response to low tones. Since variations in barometric pressure would not be communicated to the middle ear if the canal were always closed, the air pressure on the two sides of the drum would vary. This is avoided by a periodic opening of the canal which accompanies the acts of swallowing and yawning. When the throat is infected, the inflammation often spreads to this canal, which then becomes blocked, either by mucus or by the swelling of its mucous lining. The air in the middle ear is then gradually absorbed and the difference in air pressure on the two sides of the drum decreases its response to sound, and the affected ear thus becomes partially deaf.

Temporary deafness also occurs if the barometric pressure is suddenly altered by a rapid change of level (as in an aeroplane) or by the application of external pressure (as in a caisson). The deafness is, however, immediately relieved by swallowing, because the altered pressure is communicated to the other side of the drum through the opening of the Eustachian canal.

FUNCTIONS OF TYMPANUM. The function of the tympanic apparatus (consisting of drum, bones and muscles) is to transform the energy of the aerial vibrations incident on the drum into a series of mechanical movements of the plunger of the stapes, by which the pressure within the internal ear is rapidly varied. The evidence may be summarised as follows. (1) If in man the external ear be made to form a gas chamber which is connected with a manometric flame, the flame shows vibration when sound falls on the drum, which could only be caused if the drum were set into vibration. (2) If the drum be gilded, and a beam of light be made to fall on it, the excursions of the beam caused by vibrations of the drum can be recorded photographically, and are found to accompany the incidence of sound waves. (3) If to the chain of ossicles a light writing lever be attached, the point of which travels over a rotating smoked drum, when sounds fall on the drum the vibrations are recorded, showing that the ossicles are set into movement. (4) By opening the middle ear from above, and sprinkling with starch grains the ossicles lying within, the movements of the different parts can be readily followed under a low power microscope. When the drum is set into vibration by sound waves, it is readily seen that the whole chain of ossicles vibrates, so as to convey the vibrations to the plunger in the oval window. Many experimenters have noted the remarkable way in which

the apparatus responds to vibrations varying very greatly in rate. Tones of low or of high pitch appear to be recorded with equal impartiality and fidelity. Experiment therefore confirms our sensations, which show that the ear responds to vibrations varying from 40 to 30,000 per second. It is stated that the natural period of the ossicles and drum, owing to their small size, is very much more rapid even than $\frac{1}{40,000}$ second, and it is because of this that the system is able faithfully to respond to the vibrations of longer period concerned in audition.

Direct observation thus shows that the ossicles form levers which together conduct the vibrations from the drum to the plunger of the oval window. It is necessary to consider the effect of this lever system on the amplitude and force of the vibration. Motion is applied to the manubrium of the malleus and is communicated to the long process of the incus. The former is one and one-half times the length of the latter and therefore the stapes moves with two-thirds the amplitude of the drum. If the levers moved without friction, this would be accompanied by an increase in the force of the vibrations of one and one-half times. But owing to the air which surrounds the levers and thus damps their vibration, and to the energy required to set them in vibration on account of their mass, it is probable that the force of the vibrations which reaches the oval window is not more than half that incident on the manubrium. The drum, on the other hand, has an area which is about twenty times that of the oval window, and the energy incident on the drum and communicated to the manubrium is as many times greater than it would be if the sound waves were incident on the oval window direct. But owing to the energy absorbed by the levers, the magnification is probably not greater than ten times, that is one-third of the calculated amount. Two other features of the chain of ossicles should be mentioned. In the first place it will be observed that the axis, about which the malleus and incus rotate, passes through the bones, so that the big mass formed by the articular surfaces is above, and the levers below, the axis of rotation, and the ossicles are approximately balanced. Secondly, the articulation between the malleus and incus is saddle-shaped and there is a spur on the malleus which engages with the body of the incus so that, when the tensor tympani muscle relaxes and the malleus travels outwards, the spur disengages and the incus is therefore not forced to follow. When, on the other hand, the stapedius muscle is in tonic contraction, the spur is in engagement, and vibrations are therefore communicated from one bone to the other. If, however, the force applied is excessive, owing for example to a box on the ear, then the two bones separate slightly like the limbs of a compass, and the spur passes the body of the incus without communicating the blow to it. In this way rupture of the annular seal between the plunger of the stapes and the oval window is prevented.

INTERNAL EAR

Within the petrous portion of the temporal bone are two mechanisms, anatomically in close relationship, but physiologically entirely separate. One of these mechanisms, which is called the cochlea, belongs to the auditory organ ; the other, called the vestibule, consists of a series of organs which concern equilibration and have no connection with hearing.

The relation of the cochlea to the middle ear is shown in Fig. 292.

Of these structures, the cochlea alone is concerned with hearing, as the following evidence shows :

(1) Destruction of the utricle and canals causes disturbed equilibration, nystagmus and vomiting, but no deafness.

(2) Destruction of the cochlea causes deafness, but no disturbance of equilibration.

(3) Some fishes, in which no evidence of hearing can be found, possess utricle, saccule and canals, but no cochlea.

FIG. 294.
A = Middle Ear Cavity.
B = One section of Spiral duct of Cochlea.

THE COCHLEA is a tube 20 to 30 mm. long, which is spirally wound round a cone of bone called the modiolus, along the axis of which runs the auditory nerve. From the modiolus a spiral lamina of bone extends about two-thirds the way across the spiral bony canal, so as partially to divide it into two equal portions. From the outer edge of this lamina two membranes extend to the walls of the canal, so that the latter is divided throughout its length into three separate ducts. The duct nearest to the apex is called the scala vestibuli, the middle duct between the two membranes the scala media, and the duct nearest to the base the scala tympani (Fig. 295). The two membranes dividing off these ducts are quite different in structure; whereas the apical, called Reissner's membrane, is a thin layer of cells only, the basal is of complicated arrangement and is called the basilar membrane. To the latter is attached, in the organ of Corti, a series of sensitive hair cells connected with the fibres of the auditory nerve, which run through the osseous spiral lamina to the body of the modiolus. To the upper edge of the spiral lamina is attached a projecting membrane called the lamina or membrana tectoria; this is so mounted that it projects over the tips of the hair cells. Its function will be discussed later.

The way in which motion is imparted to the basilar membrane by movements of the ossicles may be described as follows. The osseous labyrinth communicates with the middle ear by means of two openings, the oval window and the circular window. The oval window connects with the upper of the three cochlear canals, *viâ* the vestibule. The upper canal is therefore called the scala vestibuli. The lower canal connects with the round window only, and since the round window is fitted with a membrane, the canal gets the name scala tympani (canal of the drum). Fitting into the oval window is the plunger of the stapes, and between the two is an annular membrane which permits motion of the plunger without allowing escape of the perilymph from the labyrinth. The circular window is also closed by its membrane, in order that leakage of perilymph may be prevented. When the plunger of the stapes is moved inwards under the influence of sound waves on the drum, the perilymph, being incompressible, is driven inwards into the vestibule. From the vestibule a corresponding volume of liquid is displaced into the scala vestibuli of the cochlea. This drives Reissner's membrane downwards, and thus increases the pressure on the endolymph in the scala media. The basilar membrane therefore moves downwards and the hair cells are drawn further away from the membrana tectoria. But this

movement of the basilar membrane presses on the fluid contents of the scala tympani which communicates with the round window, and therefore causes bulging of the membrane closing that aperture. Movement inwards of the plunger is therefore accompanied by movement downwards of the basilar

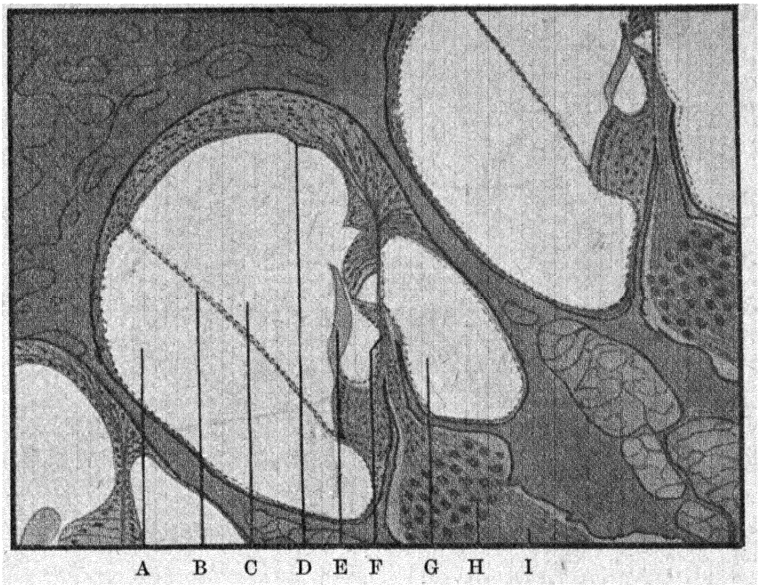

FIG. 295.

A = Scala vestibuli.
B = Reissner's Membrane.
C = Scala Media
D = Stria Vascularis.
E = Membrana Tectoria.
F = Basilar Membrane.
G = Scala Tympani.
H = Spiral Ganglion.
I = Fibres of Acoustic (Auditory) Nerve.

membrane and movement outwards of the membrane of the round window and *vice versâ*. Vibrations of the ossicles are in this way communicated to the basilar membrane and to the hair cells, causing stimulation of the auditory nerve. The basilar membrane is composed partly of strong radial fibres and partly of weak longitudinal ones. It varies in width, being narrow at the base and wide at the apex.

THE ORGAN OF CORTI. The end organ of the auditory nerve is represented by the organ of Corti, which is attached to the basilar membrane (Fig. 296). It consists of a double row of stiff cells, the inner and outer rods of Corti, which run throughout the whole length of the scala media and are surrounded by sensory epithelium, the hair cells. On the inner side of the rods of Corti there is a single row, on the outer side three rows of hair cells. The fibres of the auditory nerve pass up through the column of the cochlea, through the bipolar ganglion cells which form the spiral ganglion, and then out along grooves in the spiral lamina to end in arborisations, partly in the inner hair cells and partly among the outer hair cells. Between the hair cells are the sustentacular cells, or cells of Deiters, the peripheral processes from which join together so as to form a reticulate membrane over the hair cells, the hairs themselves projecting through orifices in the membrane. Resting on the upper surface of the membrana reticularis is the membrana tectoria. To this membrane is often ascribed a damping effect on the vibrations of the structures below. Any movement of the basilar membrane would

be transmitted to the rods of Corti, and by these to the overlying hair cells. With every vibration, these would oscillate in the line of their long axis, so that their hairs would move up and down and possibly strike against the under surface of the membrana tectoria.

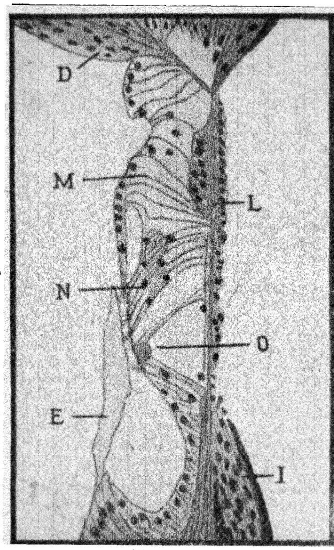

FIG. 296.

D = Stria Vascularis.
E = Membrana Tectoria.
I = Fibres of Auditory Nerve.
L = Basilar Membrane.
M = Hair Cells.
N = Hair Cells.
O = Arch of Corti.

Other views have, however, been advanced as to the way in which the hair cells become stimulated. Thus Wrightson states that there is a to-and-fro movement between the hair cells and the membrana tectoria, so that the hairs are bent first to one side and then to the other. Keith suggests, on the other hand, that the hairs are embedded in the membrana tectoria and that the stimuli are set up by the pulling of the hairs which must occur when the basilar membrane is moved. Further research is required to elucidate this point.

THE COCHLEAR MICROPHONIC ACTION. Wever and Bray observed that the ear acts like a microphone, that is, that when sounds fall on it variations of potential occur which are closely related to the incident sounds. Thus when a tone of 250 vibrations per second falls on the ear variations of potential occur at this rate in it. The potential variations are small and need to be amplified by thermionic valves in cascade, as for nervous tissues. They can be produced by tones as low as 5 cycles per second and by tones as high as 16,000. They start immediately the tone starts and stop when it stops. If the phase of the tone is suddenly changed, the phase of the microphonic potential also changes at the same instant (Hallpike, Hartridge and Rawden Smith). When the tones falling on the ear are loud, then harmonics can be detected in the microphonic potential curve. They are produced by distortion during transmission by the drumskin and ossicles (p. 481).

THE COCHLEAR TELEPHONIC ACTION. Andrief, Gersuni and Volokhov showed that alternating currents of audible frequencies are able to cause the perception of sounds when they are suitably applied to the ear.

In one frequently used arrangement the head is held on one side and the upper external ear is filled with warm saline, and into this is inserted a silver wire electrode. The other electrode is placed in the mouth or is strapped to the forearm. The electrodes are connected to a suitable A.C. source such as a wireless set or a beat oscillator. Tests with pure tones show that it is difficult or even impossible to obtain an intense response at any part of the musical scale because of the painful stimulation of the skin caused by the alternating electric current. Both low tones and high tones are poorly reproduced, there being one apparent cut off at about 50 cycles per second. Ear generated harmonics are strongly present, so much so that a pure tone is frequently mistaken for the octave above it. Short interruptions in a tone, or a sudden change of phase in a tone are observed as clearly as they are in an air borne sound (Hallpike, Hartridge and Rawden Smith).

THE CAUSE OF THE ELECTRO-ACOUSTIC EFFECTS. It seems reasonable on physical grounds to make the assumption that the microphonic and the telephonic effects of the cochlea originate in one and the same structure. Just as in the piezo-electric effect: When a suitable crystal is moved it generates an electric potential, or when a suitable potential is applied to it a movement is brought about, so in the ear, a structure or structures are present, movements of which generate electrical potentials, or when potentials are applied from without, movements are caused which bring about the sensations of sound. What the structure or structures are is not known at the present time, the hair cells of Corti's organ have been suggested, so also has Reissner's membrane.

2. THE THEORIES OF HEARING

THE RESONANCE THEORY (of Helmholtz) states that the organ of Corti and the basilar membrane together form a series of automatically recording resonators. In the same way that each of the strings of a piano can be set into vibration by the sounding of a note which corresponds with it in pitch, so also can the different fibres of the basilar membrane vibrate to a certain note, and so cause stimulation of the hair cells which are attached to it.

Four objections have been made to this theory. (1) That the fibres of the basilar membrane are so short that they could not respond to the low notes which the ear is able to hear. The answer to this criticism is that not only the length, but also the tension and weight of a cord determine its vibration rate. In the case of the basilar membrane the tensions in the fibres are probably minute, while the weights of the arches of Corti and the hair cells must make the period of vibration so much the longer.

(2) That the separate fibres of the basilar membrane are bound together so that vibration of the separate fibres would be impossible. This objection was met by the calculation, by Helmholtz, that a uniform membrane, in which the tension was greater from side to side than longitudinally, would be able to respond in the manner required.

(3) That the difference in length of the fibres is not sufficiently great for the short ones to vibrate to notes of 4,000 vibrations per second, while the long ones vibrate to 40 vibrations per second only. This objection also fails when we reflect that not only length, but also tension and weight determine the period of vibration of a stretched cord. However accurately we can determine length and weight by histological examination, the method tells us nothing concerning tension. This objection therefore does not prevail.

(4) That if the cochlea depends for its action on the resonance of the basilar fibres, we should expect a musical note to seem to go on sounding after the note has actually ceased. Since, on the other hand, we know from our own experience that words such as ' utter,' in which there is an interval of silence between the two ' ts,' are quite different from ' udder,' in which there is no interval of silence, it follows that the fibres of the basilar membrane have not been in vibration for long after the sound ceased, and therefore probably resonance of the basilar membrane is slight. But if we suppose the fibres to be highly damped, so that they come to rest at once when the note ceases, how comes it that they can be set in motion so readily that in only three or four vibrations a note is distinctly heard? The answer is

probably obtained from the fact that the cochlea is filled with liquid. This liquid makes the basilar membrane 'dead beat,' because movements, when the liquid is still, set up eddies which, owing to the viscosity of the liquid, quickly check the motion. On the other hand, when a sound is entering the ear, and the fluid is therefore in motion, this movement is the more rapidly imparted to the basilar membrane because of its continuity, but even if it consisted of separate fibres it would still be set promptly into vibration owing to the viscosity of the liquid. In this way one can explain both the rapid response and the rapid damping of the cochlea.

In favour of Helmholtz' theory we have the following evidence :

(1) In boiler-makers' disease we have inability to hear high notes, and we find that it is the short fibres of the basilar membrane which have degenerated.

(2) In experiments in which the ears of animals have been stimulated for long periods by the same note, subsequent examination has shown the localisation of degeneration to one part of the organ of Corti. With a high note the short fibres are affected, with a low note the long.

(3) If one of the ears be fatigued by prolonged stimulation to a constant note, its response to the same note is found to be inhibited, but notes of slightly longer or slightly shorter pitch are found to be unaffected. This shows clearly that the response to a given rate of vibration must only affect a certain limited number of hair cells and nerve fibres, and is therefore strongly in favour of Helmholtz' theory.

(4) Animals whose calls have a small range of pitch (*e.g.* birds) have short basilar membranes which vary but little in width.

(5) Animals in which different parts of the cochlea have been destroyed, appear to give definite evidence for deafness to high notes when the short basilar fibres are damaged, and deafness to low notes when the long fibres have been removed.

(6) Patients are found in whom there are islands of deafness, that is, they are deaf to a limited part of the musical scale. The Helmholtz theory readily explains these cases as being due to local disease of certain basilar fibres or their corresponding hair cells. Further, there are cases in which the two ears give different notes, a condition called double disharmonic hearing. This is easily explained by a change in the natural period of the fibres of the basilar membrane in the diseased ear, as the result either of stretching or of increased mass due to inflammation.

(7) If the input leads of a sensitive valve amplifier be connected to the cochlea of an anæsthetised animal, then, as Wever and Bray showed, the incidence of musical tones on the ear is accompanied by potential fluctuations in the cochlea at a frequency corresponding to that of the musical tone. Hallpike and Rawden Smith found, moreover, that with low-pitched tones the potential fluctuations were greater near the apex of the cochlea, whereas, with high-pitched tones they were greater near its base, as should be the case on the resonance hypothesis.

(8) When a musical tone, transmitted by a telephone (*i.e.* an aperiodic) circuit, is suddenly changed in phase by a half-cycle the potential fluctuations in the circuit continue almost without interruption.

When a musical tone, which is causing a tuned structure to perform resonant vibrations, is suddenly changed in phase by a half-cycle the vibrations of the tuned structure are first arrested and are then re-established. On performing a similar experiment with the human ear, Hartridge found that just after the change of phase the musical tone is heard to undergo a short interruption, *i.e.* the ear, unlike a telephone, behaves as if it contained resonators. This phase-change effect was heard whether the experiment

was done in an ordinary room where there are echoes or in a room with sound-absorbing walls in which echoes were reduced to a minimum; the effect cannot therefore be due to them.

(9) When a musical tone is interrupted for short periods of time similar interruptions can be identified in a telephone circuit which is excited by the sound. When a musical tone which is causing a tuned structure to perform resonant vibrations is interrupted for a short period of time the vibrations of the tuned circuit are found to continue with only a small diminution in amplitude. On performing a similar experiment with the ear it is found that short interruptions cannot be detected, *i.e.* the ear again behaves as if it contained resonators.

(10) When two musical tones differing considerably in frequency, for example, 200 v. per sec. and 250 v. per sec., are sounded simultaneously the voltage fluctuations in a telephone circuit are found to undergo periodic increases and decreases, which correspond to beats between the two musical tones. When the above two tones are caused to set up vibrations in a graduated series of resonators, two groups of resonators are found to be set into vibration, one by each tone. A careful examination of the groups shows that neither of them is exhibiting appreciable beats. On performing the experiments with the ear no beats are to be observed, *i.e.* the ear behaves as a series of resonators.

(11) When two musical tones having frequencies in the ratio of 1 to 3 are sounded simultaneously the voltage fluctuations in a telephone circuit excited by them are found to be greatly affected by the relative phases between the two tones. In one phase relationship a single sharp peak curve is produced. In another phase relationship a double hump curve is produced. When the above two tones are caused to set up vibrations in a graduated series of resonators two groups of resonators are found to be set into vibration, one group for each tone. The careful examination of the groups shows that neither their frequency nor amplitude is affected appreciably by changing the relative phases of the two tones. On performing the experiment with the ear it is found that changing the relative phases of the tones has no effect on the sounds which are produced, *i.e.* the ear behaves in accordance with the resonance theory and contrary to the telephone theory.

(12) It is well known that resonators exhibit the properties of selectivity (sharpness of resonance) and persistence, and that both these properties are related to the frictional losses to which the resonators are subject. In consequence of this, when different degrees of selectivity are multiplied by corresponding degrees of persistence a constant value is obtained, as is shown in the following table :—

S (selectivity)	P (persistence)	S × P
1·5	40	60
2·0	30	60
3·0	20	60
4·0	15	60
6·0	10	60

(Selectivity in the above table = percentage difference of frequency causing 1/10 the amplitude of that caused by the intune frequency at the same energy level.)

(Persistence in the above table = number of vibrations which correspond to the time required for the amplitude to be reduced to 1/10 its initial value.)

Now the selectivity of a graduated series of resonators can be ascertained experimentally by finding the smallest difference of frequency which causes beats owing to the overlap of the two vibrating groups of resonators. The persistence of a series of resonators can be ascertained experimentally by finding the duration of the longest interruption in the incoming sound energy which produces no observable decrease in amplitude. Both these methods of research are applicable to the ear, and the table below gives the values I obtained. The last column shows that their product gives a reasonably constant value, as should be the case if the ear resonators are of the kind familiar to the physicist.

Tone	S	P	S × P
128	12·7	1·78	22·6
256	10·0	2·06	20·6
326	9·45	2·19	20·7
384	9 07	2·18	19 75
512	8·45	2·37	20 0
640	8·15	2·54	20·65
760	7·82	2·68	20 95
1024	7 22	3 01	21·70

It would appear, therefore, that the evidence in favour of Helmholtz' theory is very convincing. Other hypotheses have been proposed, however, two of which will now receive brief consideration.

The Telephone Theory (of Rutherford) compares the cochlea with a telephone. In the same way as the diaphragm of the receiver is set into vibration by the sound waves, and starts corresponding variations in the strength of the current conducted to the transmitter, so the vibration of the basilar membrane as a whole causes impulses to be sent up the auditory nerve which correspond with the air vibrations received by the ear. Analysis does not take place in the cochlea at all, but in the brain. Wrightson, who has restated this theory and added much detail to it, states that the cerebral analysis is effected by differences between the time intervals of the points of zero pressure and of the maximum plus and minus pressures.

The following objections may be stated against the Rutherford-Wrightson hypothesis:

(1) It assumes that the auditory nerve can conduct complicated wave forms, intact as to pitch and amplitude, at rates up to 15,000 vibrations per second. Rutherford, in this connection, pointed to the motor nerves of the bee's wing, which are capable of responding to transmitted impulses at 460 per second. Between 15,000 and 460 is, however, a big gap which will certainly have to be bridged before this view as to the transmission of the vibrations intact to the brain can be accepted.

(2) We cannot picture a cerebral apparatus which can analyse these complicated nerve impulses even if they could reach it, and neither Rutherford nor Wrightson assist us to do so. The relegation of the powers of analysis to the cerebral cortex is, at the present at any rate, equivalent to giving up any attempt to explain the power of analysis possessed by the organ of hearing.

(3) It would seem that a very much simpler organ than that of the cochlea would be sufficient to convert sound waves into nerve impulses, if no analysis of the stimulus took place there.

(4) It would be very difficult to explain, on this hypothesis, the localisation of deafness to certain notes, which accompanies disease of part of the organ of Corti.

(5) This hypothesis does not explain why fatigue to one note leaves the response to all other notes apparently unaffected in intensity.

The objections to this hypothesis are therefore of a formidable character. Much additional evidence in its favour would be necessary to place it even on a par with the theory of Helmholtz.

The Sound Pattern Theory (of Waller and Ewald), stated that the basilar membrane vibrated in the form of pressure patterns, which are similar to those which may be seen on a vibrating plate. Ewald found by experiment that the patterns take

he form of equidistant stationary nodes or ridges, the distance between which varies rith the pitch of the note entering the mechanism. The distance between the nodes is neasured by the hair cells, and corresponding impulses are sent to the auditory centre. 'he advantage of this hypothesis is, that like that of Helmholtz, it places the analysis of he sound waves in the cochlea and therefore does not, like Rutherford's hypothesis, equire the transmission by the auditory nerve of rapid impulses or the analysis of such mpulses by the brain. It is clear, however, that so far as our present knowledge goes the vidence is all in favour of the Helmholtz view.

THE VOLLEY THEORY has been devised to explain two different facts: (1) that he position in space of sources emitting tones below about 1,000 cycles is identified y the recognition of the time interval between the arrival of the sound at the two ars. When the source is straight in front of the observer the sound reaches the right nd left ear simultaneously. If this source is moved round to the right the sound eaches the right ear first. If the brain is to appreciate this fact the nerve impulses eaching it must be discontinuous and must consist of volleys having time relationships orresponding to the vibrations set up in the ear by the sound waves. Thus a tone of 00 vibrations per second from a source of sound 45° right of the observer will cause 00 volleys of nerve impulses to travel to the brain along each auditory nerve. But he sound waves to the left ear have to travel about 4 inches further than those to the ight, and the velocity of sound in air being 13,200 inches per second, this distance will ake $\frac{1}{3,300}$ of a second and the volleys up the right auditory nerve will be that time

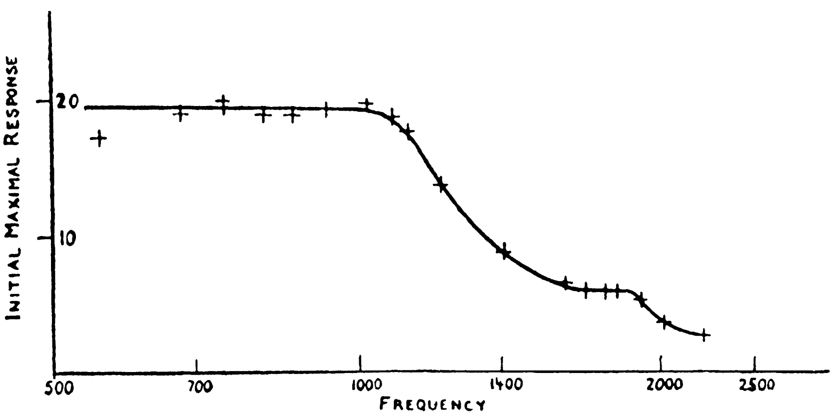

FIG 297. Shows the relationship between the electrical response of the auditory nerve and frequency in cycles per second (Derbyshire and Davis, *Amer. J. Phys.*) Between 500 and 1,000 cycles the curve is level because all the auditory nerve fibres respond to every vibration of the basilar membrane. The bend in the curve that occurs at 1,000 cycles is due to the fact that above this frequency, half the nerve fibres are responding to every other vibration in accordance with the volley theory. The bend in the curve that occurs at a frequency of 1900 cycles is due to the fact that above this frequency, one-third of the nerve fibres are responding to every third vibration.

ahead of those up the left nerve. (2) That the responses in the auditory nerve are found to correspond in frequency to those of the tones falling on the ear up to about 2,200 cycles per second. But the separate fibres of the auditory nerve, like those of other nerves which have been experimentally investigated, cannot transmit more than about 1,000 impulses per second. The volley theory explains the faster rate of transmission by supposing that each fibre responds to every other sound wave, and possibly when the fastest rate of all is being transmitted (2,200 cycles per second), each fibre of the auditory nerve may respond to every third sound wave. When, however, tones of higher pitch are falling on the ear and each of the auditory nerve fibres should be transmitting every fourth, fifth, sixth, etc., up to possibly every tenth wave, the transmission of the nerve impulses has become too irregular for the precise conditions to be experimentally identified. If all the auditory nerve fibres had precisely the same refractory phase, and

the same rate of conduction, then proof of the volley theory could probably be obtained for all musical tones including the highest. But this seems not to be the case. That the volley theory holds good for tones below about 2,400 cycles per second was shown by the experiments of Derbyshire and Davis. In Fig. 297 is shown the relationship between the frequency of the tone and the size of the electric response of the auditory nerve of a cat taken immediately after the brief transcent (the "on effect") which always occurs at the beginning of a musical tone. It will be seen that the response is a maximum from 500 to 1,000 cycles per second. Experiment shows that this maximum extends right down the musical scale to low tones. Above 1,000 cycles per second the graph shows a fall so that at 1,400 cycles per second the response has half the maximum value. At about 1,600 cycles the nerve response has flattened out and at 1,800 undergoes a second abrupt fall. The first fall at 1,100 cycles occurs at a point where, on the volley theory, a fall would be expected because some of the auditory nerve fibres are beginning to respond to every other wave. The second fall at about 1,800 cycles occurs at a point where on the volley theory a further fall would be expected, because some of the auditory nerve fibres are failing to be able to respond to every other wave and are beginning to respond to every third wave.

3. THE PHENOMENA OF HEARING

Limits of Pitch. The ear is unable to perceive a tone the pitch of which falls above or below certain fairly well-defined limits. If the number of vibrations is less than about twenty per second no musical tone is produced, the individual vibrations being perceived as a series of pulses in the surrounding air, and it is only when we increase the number to about thirty per second that we are able to appreciate a pitch in the note produced. As the number of vibrations per second is increased the note rises steadily without break till we arrive at about 15,000 vibrations per second. Above this number of vibrations, the human ear is incapable of perceiving any note at all, though it is probable that small animals can hear notes still higher in the scale. In music neither the lowest nor the highest tones are used. The lowest tone of large organs, 16 vibrations per second, is that produced by the sixty-four foot pipe, and one can hardly speak of its effect as that of a musical tone. The highest notes employed in music are *a*4 and *c*5 with 3520 and 4224 vibrations per second on the piano, and *d*5 with 4752 vibrations on the piccolo flute. In music therefore we only employ between 25 and 4800 vibrations per second, *i.e.* about seven and a half octaves.

Beats and Dissonance. If we sound two tuning forks, the vibrations of which differ only by one or two per second, the phenomenon known as 'beats' is produced. This is due to the summation and interference of the waves from the two tuning forks. Let us suppose we have tuning forks vibrating, one at 100 and the other at 101 times a second, and that they begin vibrating together. At first the waves of compression started by each fork will coincide, so that the total compression of the air at each beat will be the compound effect of the compression produced by the two forks, which will reinforce one another. After the lapse of half a second the tuning forks will be at different phases of their excursion. The 101 fork will be moving in one direction while the 100 fork is moving in the other, so that the compression produced by one fork coincides with the expansion of the air produced by the moving backwards of the other fork. The sound produced by one fork is therefore diminished by the sound produced by the other fork, and the total sound is less than that of either of the two forks. At the end of one second, the phases of the two forks once more corresponding, we shall get the sound increased in loudness; thus there is an alternate waxing and waning of the sound which recurs once a second and is spoken of as a 'beat.'

The number of beats per second may be used to determine the differences in the vibration frequencies of two forks. Thus, two forks vibrating, one at 100 and the other at 110, will give ten beats per second. As the number of

beats increases, the effect produced on the ear becomes more and more disagreeable, just as the rapid alternation of illumination produced by a flickering light is disagreeable to the eye. This objectionable character of the sound is most marked when the beats recur at about thirty-three times per second ; the individual beats are not then distinguished, but we speak of the sound as discordant or *dissonant*.

COMBINATION TONES. If two tuning forks, with an interval of one-fifth between them, are sounded together, we may hear a weak lower tone, the pitch of which is an octave below that of the lower fork. This is known as a 'combination tone.' The combination tones are divided into two classes : (1) 'difference tones,' in which the frequency is the difference of the frequencies of the generating tones ; (2) 'summation tones,' which have a pitch corresponding to the sum of the vibrations of the tone of which they are composed. By means of appropriate resonators these tones can be reinforced, showing that they have an objective existence. They are, however, reinforced in the ear by the asymmetry of the drum skin and ossicles.

In consequence of these combination tones the sound spectrum of the motion conveyed to the cochlea is much richer in the higher frequencies than is the wave motion incident on the drum.

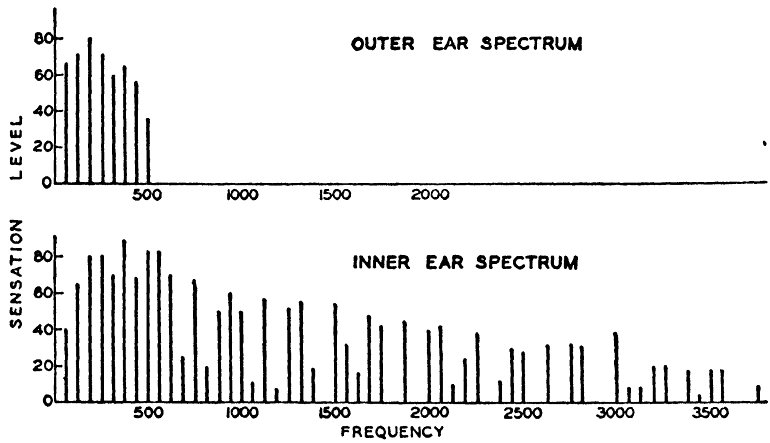

FIG. 298. Loudness spectrum : The top graph shows actual spectrum of a note of an organ pipe impressed upon the outer ear. The lower one gives estimated spectrum as impressed upon the nerve endings of the cochlea. The additional frequencies introduced owing to rectification effected by the drum are well shown. (From HARVEY FLETCHER, "Speech and Hearing," Macmillan & Co., London, 1929.)

CONSONANCE. The opposite condition of *consonance* or harmony involves therefore, in the first place, an absence of beats, *i.e.* of rhythmic oscillations of amplitude of sound waves which reach the ear. The most complete consonance is attained when the two notes which are sounded together are identical in frequency. Almost as complete is the consonance obtained when a note is sounded together with its octave. The octave and other consonant intervals which are employed in music are as follows :—

1 : 2	Octave
2 : 3	Fifth
3 : 4	Fourth
4 : 5	Major third
5 : 6	Minor third
5 : 8	Minor sixth
3 : 5	Major sixth

It will be noticed that in all these consonant combinations, the vibration frequencies of the notes are in proportion to small whole numbers. If we put down not only the fundamental tones of these notes, but also their overtones, we shall see that there is considerable identity as regards the latter. In the case of the octave the two are almost identical, the only difference being the ground tone of the lower note; but the identity diminishes as we pass from the octave through the thirds to the sixths. The overtones which are identical are shown by black type:

Overtone

$\frac{1}{2}$		**1**		**2**		3		**4**		5		**6**		7		**8**		9		**10**	
		D		**2**		S		**4**				**6**				**8**				**10**	
$\frac{2}{3}$		2		4		**6**		8		10		**12**		14		16		**18**		20	
	D		3		S	**6**			9			**12**			15			**18**			
$\frac{3}{4}$		3		6		9		**12**		15		18		21		**24**		27		30	
	D		4		S 8			**12**			16		20			**24**			28		
$\frac{4}{5}$		4		8		12		16		**20**		24		28		32		36		**40**	
	D		5		S 10		15			**20**			25		30		35			**40**	
$\frac{5}{6}$		5		10		15		20		25		**30**		35		40		45		50	
	D		6		S 12		18		24			**30**			36		42		48		
$\frac{3}{5}$		3		6		9		12		**15**		18		21		24		27		**30**	
	D		5		S		10			**15**			20				25			**30**	
$\frac{8}{9}$		8		16		24		32		40		48		56		64		**72**		80	
	D		9		S 18		27		36		45		54		63			**72**			
$\frac{8}{15}$		8		16		24		32		40		48		56		64		72		80	
	D	15			S		30				45				60				75		90

D = difference tone. S = summation tone.

It will be observed that in all cases, with the exception of the octave, the summation tone is discordant with the other partial tones.

In the second $\left(\frac{8}{9}\right)$ and the seventh, both of which are discordant, it is only the eighth and ninth overtones which are identical, while the fundamental tones will as a rule be so close together that beats of a number calculated to give dissonance will be produced. Since the phenomenon of beats depends on the absolute number of vibrations per second, they are more easily produced by two notes near together at the lower end of the scale than at the upper end. Thus the dissonance is quite perceptible in a major third at the lower end of the piano, but disappears at the upper part, since here the beats produced are so rapid that they become imperceptible.

The various notes used in music are obtained by employing the consonant intervals which we have given above. The major chord is composed of the fundamental tone, the major third and the fifth. If we take '*c*' as the fundamental tone, the notes of the chord are *c*, *e*, *g*, with vibration frequencies corresponding to 1, $\frac{5}{4}$, $\frac{3}{2}$, *i.e.* 4, 5, 6. The major chord from *g* is *g*, *b*, *d*, *i.e.* three notes with vibration frequencies corresponding to $\frac{3}{2}$, $\frac{15}{8}$, $\frac{9}{4}$, *i.e.* 4, 5, 6. The major chord from the fourth, *f*, is *f*, *a*, *c*, with the vibration frequencies $\frac{4}{3}$, $\frac{20}{12}$, $\frac{12}{6}$, *i.e.* 4, 5, 6. The C major scale is therefore as follows:

C	D	E	F	G	A	B	C	
1	$\frac{9}{8}$	$\frac{5}{4}$	$\frac{4}{3}$	$\frac{3}{2}$	$\frac{5}{3}$	$\frac{15}{8}$	2	**Major scale**
1	$\frac{9}{8}$	$\frac{6}{5}$	$\frac{4}{3}$	$\frac{3}{2}$	$\frac{8}{5}$	$\frac{15}{8}$	2	**Minor scale.**

Different instruments are tuned to one normal note, *i.e.* to A with 440 vibrations per second (this note varies somewhat in different countries). Taking this as the normal, the vibration frequencies of the various notes used in music are given in the following Table :—

Notes	Vibrations per second.						
C. . . .	33	66	132	264	528	1056	2112
D. . . .	37·125	74·25	148·5	297	594	1188	2376
E. . . .	41·25	82·5	165	330	660	1320	2640
F. . . .	44	88	176	352	704	1408	2816
G. . . .	49·5	99	198	396	792	1584	3168
A. . . .	55	110	220	440	880	1760	3520
B. . . .	61·875	123·75	247·5	495	990	1980	3960

SOUND ANALYSIS. Not only can the ear appreciate differences between different musical instruments, dependent on the varying overtones present in the sound produced by each instrument but, when a number of these instruments are sounded simultaneously, the ear can pick out from the compound sound the notes due to an individual instrument; and a person with a trained ear can with ease name notes composing any chord struck on an instrument such as the piano.

This power of analysis, which is possessed by the auditory apparatus, may be stated in the form of a law, known as Ohm's law :

"Every motion of the air which corresponds to a composite mass of musical tones is capable of being analysed into a sum of simple pendular vibrations, and to each single vibration corresponds a simple tone, sensible to the ear and having a pitch determined by the periodic time of the corresponding motion of the air."

SOUND LOCALISATION. Experiment shows that man and animals can appreciate with fair accuracy the direction from which a sound is coming. There has been considerable speculation as to how this information is obtained and, although the subject has not been completely elucidated, it appears to have been established that the following factors are important :—

(1) The intensity of the sounds entering the two ears. When a sound is coming from one side, the ear on that side receives the more powerful stimulus.

The relative intensities of the components of high and low pitch vary with direction, because the notes of long wavelength (low pitch) will be diffracted the more readily round the head to the ear away from the sound than will those of short wavelength (high pitch).

(2) The sounds reaching the nearer ear will arrive earlier than those stimulating the other, because of the time taken to travel round the head. The nerve impulses from the two ears will therefore not arrive at the same instant, and by an appreciation of the difference in time, the approximate position of an external object can be gauged.

That this factor is of great importance can be shown by experiment in the following manner : A stethoscope with two earpieces is fitted in position, and to its mouthpiece is applied a loud tuning fork. The tube connecting the mouthpiece to one of the earpieces has an adjustable U-piece like a trombone, so that the distance travelled by the sound in reaching that ear can be varied. The other tube has a length which is equal to that of the others, with the U-piece in its mid position. When a note is sounded and the U-piece altered, the position of the sound appears to move from one side to the other according to which ear has the shorter tube.

In everyday life both the intensity and the time factors play their part. The intensity factor is more important for frequencies above 1,000 v. per sec., the time factor being more important for frequencies below this. Thus in the case of a musical tone of 400 v. per sec. rich in harmonics, the fundamental (400) and first harmonic (800) would involve the time factor while the higher harmonics would be bringing in the intensity factor.

(3) In animals, the ability to turn the ears in different directions and so find the direction of maximum intensity must be of the utmost possible value in sound localisation.

Note on the Decibel The DECIBEL is a physical unit in terms of which DIFFERENCES IN SOUND INTENSITIES can be expressed. 10 DECIBELS = 1 BEL, called after Alexander Graham Bell, who invented the telephone. The decibel scale is logarithmic. INTENSITY DIFFERENCE BETWEEN TWO SOUNDS IN DECIBELS =

$$10 \times \log. \frac{\text{physical power of 1 sound}}{\text{physical power of other sound}}$$

e g., sound 1 : 2	=	3 decibels.
1 : 5	=	7 ,,
1 : 100	=	20 ,,
1 : 1,000	=	30 ,,

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4. SPEECH

The development of the analytical powers of the auditory apparatus is closely connected with that of the faculty of speech, which is best considered with the central nervous system. We may first consider the mechanism of production of *voice*, which man shares with many other animals, before discussing the physiology of the wholly human faculty of speech.

Voice is produced in the larynx, a modified portion of the wind-pipe, by the vibrations of two elastic bands, the vocal cords, which are set into action by an expiratory current of air from the lungs. In many respects the larynx resembles a reed instrument, in which a current of air is caused to vibrate by the vibrations of an elastic tongue. Whereas, however, the period of the vibrations in such an instrument, and therefore the note, is determined by the length of the tube which is attached to the reed and by the lengths of the reeds themselves, in the larynx the note produced by the blast of air is modified partly by alterations in the tension of the vocal cords, partly by altering the size of the aperture and partly by varying the strength of the blast of air.

ANATOMICAL MECHANISM OF THE LARYNX. The essential framework of the larynx is formed by four cartilages, viz. the cricoid, the thyroid, and the two arytenoid cartilages (Fig. 299). The cricoid cartilage, which lies immediately over the uppermost ring of the trachea, is shaped like a signet ring, the small narrow part being directed forwards and the broad plate backwards. The thyroid cartilage consists of two parts or alæ, joined together in front and forming the prominence known as Adam's apple ; behind, it presents four processes or cornua, the superior of which are attached

by ligaments to the hyoid bone, while the inferior cornua articulate with the postero-lateral portion of the cricoid cartilage. By means of this articulation very free movement is permitted between the two cartilages, the general direction of movement being one of rotation of the cricoid cartilage on the thyroid, round a horizontal axis directly through the two articular surfaces between the two cartilages, while movements of the thyroid upon the cricoid are also possible in the upward, downward, forward, and backward directions. The two arytenoid cartilages are pyramidal in shape. By their bases they articulate at some distance from the middle line with convex articular surfaces

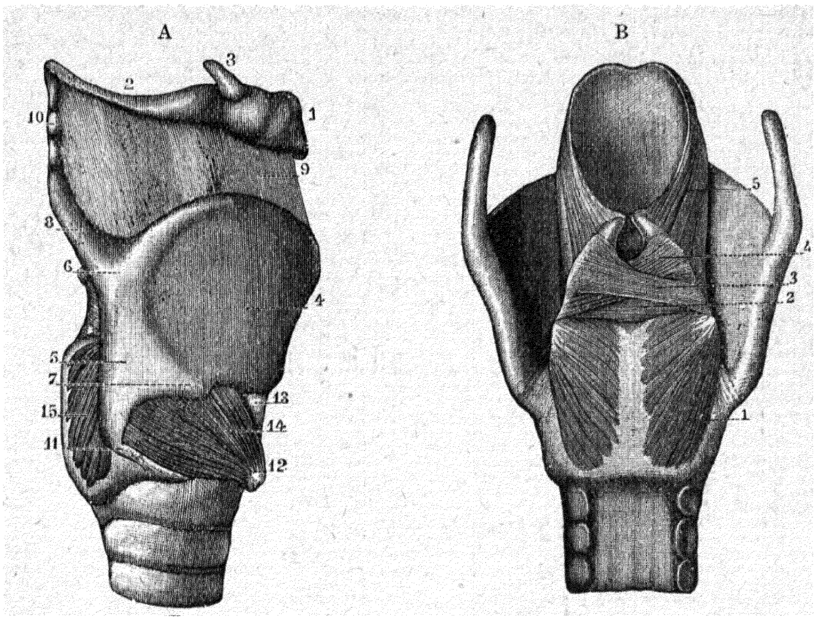

FIG. 299. Muscles of the Larynx. (SAPPEY.)

A, as shown in a view of the larynx from the right side.

1. Hyoid bone.
2, 3. Its cornua.
4. Right ala of thyroid cartilage.
5. Posterior part of the same separated by oblique line from anterior part.
6, 7. Superior and inferior tubercles at ends of oblique line.
8. Upper cornu of thyroid.
9. Thyro-hyoid ligament.
10. Cartilago triticea.
11. Lower cornu of thyroid, articulating with the cricoid.
12. Anterior part of cricoid.
13. Crico-thyroid membrane.
14. Crico-thyroid muscle.
15. Posterior crico-arytenoid muscle, partly hidden by thyroid cartilage.

B, as seen in a view of the larynx from behind.

1. Posterior crico-arytenoid.
2. Arytenoid muscle.
3, 4. Oblique fibres passing around the edge of the arytenoid cartilage to join the thyro-arytenoid, and to form the aryteno-epiglottic, 5.

situated in the upper margin of the plate of the cricoid cartilage. The anterior angle of the base is the vocal process, while the external angle is the muscular process of the arytenoid. The crico-arytenoid joints permit of two kinds of movements of the arytenoid cartilages :

(1) Rotation on their base around their vertical long axis, so that the anterior vocal process is rotated outwards and the muscular process backwards and inwards, or conversely.

(2) Sliding movements of the whole arytenoid cartilage either outwards or inwards, so that their inner margins may be drawn apart or approximated.

The larynx is covered internally by a mucous membrane continuous with that of the trachea. It is lined with ciliated epithelium, except over the vocal cords, where the epithelium is stratified. The two vocal cords, or thyro-arytenoid ligaments, consist of elastic fibres which run from the middle of the inner angle of the thyroid cartilage to be inserted into the anterior angle of the arytenoid cartilages. Their length in man

is about 15 mm., in woman about 11 mm. The cleft between them is known as the glottis or *rima glottidis*.

Two ridges of mucous membrane above and parallel to the vocal cords are the false vocal cords. Between the true and the false vocal cords on each side is a recess known as the ventricle of Morgagni. This ventricle permits the free vibration of the vocal cords. The false cords take no part in phonation, but help to keep the true cords moistened by the secretion of the numerous mucous glands with which they are provided. The false cords are also used in holding the breath. For this purpose they function in a similar manner to the mitral valve of the heart. It is found that animals, who need the thorax to be fixed in order that they may climb or strike, have well developed false cords. The position and tension of the vocal cords are determined by the action of the intrinsic muscles of the larynx. The part taken by the various muscles in each movement cannot be directly ascertained. We can in most cases study only the direction of the fibres and judge, from this direction and consequent isolated action of the muscles, the part taken by any given muscle in the production of voice. The chief muscles (Fig. 299) are the following:—

(1) The *crico-thyroid* muscle is a short triangular muscle attached below to the cricoid cartilage and above to the inferior border of the thyroid cartilage: the fibres pass from below upwards and backwards. When this muscle contracts, the cricoid cartilage is drawn up under the anterior part of the thyroid cartilage, so that its broad expansion behind, with the arytenoid cartilages, is drawn downwards and backwards, thus putting the vocal cords on the stretch. This muscle is probably the most important in determining the tension of the vocal cord.

(2) The *posterior crico-arytenoid* muscle arises from a broad depression on the corresponding half of the posterior surface of the cricoid cartilage. It passes upwards and outwards, its fibres converging, to be inserted into the outer angle of the arytenoid cartilage. These muscles rotate the outer angle of the arytenoid cartilages backwards and inwards. They thus cause a movement outwards of the anterior angles, so that the glottis is widened. During every act of inspiration there is a widening of the glottis, which is probably effected by contraction of these muscles. If they are paralysed the vocal cords are approximated and tend to come together during inspiration, so that dyspnœa may be produced.

(3) The *lateral crico-arytenoid* muscle arises from the upper border of the cricoid cartilage and passes backwards to be inserted into the muscular process of the arytenoid cartilage. These muscles when they contract pull the muscular process of the arytenoid cartilage forwards and downwards, thus approximating the vocal cords at their posterior ends and antagonising the action of the posterior crico-arytenoid muscles.

(4) The *arytenoid* muscles consist of transverse fibres, some of which decussate, uniting the posterior surface of the two arytenoid cartilages. When they contract they draw the arytenoid cartilages together.

(5) The *thyro-arytenoid* muscles consist of two portions. The outer fibres rise in front from the thyroid cartilage and pass backwards to be inserted into the lateral border and the muscular process of the arytenoid cartilage. Some of the fibres pass obliquely upwards towards the aryteno-epiglottidean folds. These are often spoken of as a separate muscle, the *thyro-epiglottidean*. By their action they tend to draw the arytenoid cartilages forwards and to relax the vocal cords. The upper fibres may also assist in depressing the epiglottis. The inner fibres are called the *musculus vocalis*. They arise from the lower half of the angle of the thyroid cartilage, and, passing backwards in the vocal cords, are attached to the vocal processes and to the adjacent parts of the outer surfaces of the arytenoid cartilages. Many fibres do not run the whole distance, but end in an attachment to some part of the vocal cord. Although their action must be to draw the arytenoid cartilages forwards, yet, since they are contained in the vibrating portion of the vocal cords, they cannot by their contraction relax these cords. It is probable that they play a great part in determining the tension of the vocal cords after these have been put on the stretch by the action of the crico-thyroid muscles. They may possibly act as a sort of fine adjustment of the tension, the coarse adjustment being represented by the crico-thyroids.

THE PRODUCTION OF VOICE

In order to study the changes in the larynx which are associated with voice production, we must make use of the *laryngoscope*. The principle of this instrument is very simple. A large concave mirror with a central aperture is fixed before one eye of the observer, sitting in front of the patient or person to be observed. The latter is directed to throw his head slightly

backwards and to open his mouth. In order to keep the tongue out of the way the patient is made to hold the end of it by means of a towel. The mirror is then so arranged as to reflect light from a lamp into the cavity of the mouth. A small mirror fixed in a handle is then warmed, so as to prevent the condensation of the patient's breath, and passed to the back of the mouth until it rests upon and slightly raises the base of the uvula.

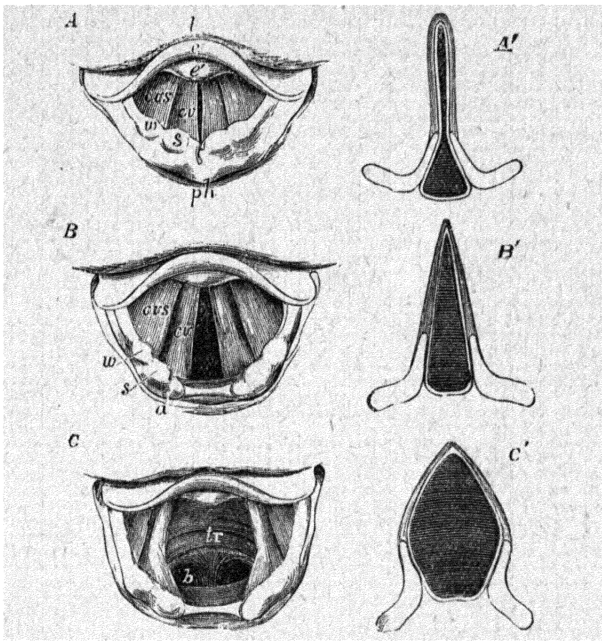

FIG. 300. Three Laryngoscopic Views of the Superior Aperture of the Larynx and surrounding parts in different states of the Glottis during life. (From CZERMAK.)

A, the glottis during the emission of a high note in singing.
B, in easy or quiet inhalation of air.
C, in the state of widest possible dilatation, as in inhaling a very deep breath.

A′, B′, C′ show in horizontal sections of the glottis the position of the vocal ligaments and arytenoid cartilages in the three states represented in the other Figures. Letters indicate as follows: *l*, base of tongue; *e*, upper free part of epiglottis, *e′*, tubercle of epiglottis; *ph*, part of anterior wall of pharynx behind the larynx; in the margin of the aryteno-epiglottidean fold *w*, the swelling caused by the cuneiform cartilage; *s*, that of the corniculum; *a*, tip of arytenoid cartilages; *cv*, true vocal cords; *cvs*, false vocal cords; between them the ventricle of the larynx.

In C, *tr* is placed on the anterior wall of the trachea, and *b* indicates the commencement of the two bronchi.

By this mirror the light reflected into the mouth from the large mirror is again reflected down on to the larynx, and a reflection of the larynx and trachea is seen in the mirror.

By laryngoscopic examination we can see the base of the tongue, behind which is the outline of the epiglottis. Behind this again in the middle line are seen the two vocal cords, white and shining (Fig. 300). The cords appear to approximate posteriorly; between them is a narrow chink, the diameter of which varies with each respiration, being wider during inspiration. On each side of the true vocal cords are seen the pink false vocal cords. In some cases the rings of the trachea, and even the bifurcation of the trachea itself (Fig. 300, C), may be seen in the interval between the vocal cords.

In order that the vocal cords may be set into vibration, they must be put into a state of tension, and the aperture of the glottis narrowed so as to afford resistance to the current of air. In the dead larynx it is possible to produce sounds by forcing air from bellows through the trachea, after the vocal cords have been put on the stretch by pulling the arytenoid cartilages backwards. By experimenting on patients on whom tracheotomy has been performed, it has been found that the pressure of air in the trachea, necessary to cause production of voice, is, for a tone of ordinary loudness and pitch, between 140 and 240 mm. of water, and with loud shouting the pressure rises to as much as 945 mm. of water. This pressure is furnished by the contraction of the expiratory muscles, *i.e.* of the abdomen and of the thorax. Since the pitch of the note produced rises with increasing force of the blast, while the tension of the cords remains constant, it is evident that, in the act of 'swelling' on a note, the increased pressure necessary for the crescendo must be associated with diminishing tension of the cords. It is the failure to secure this muscular relaxation that so often causes a vocalist to sing sharp when swelling on any given note.

The voice, like the sound produced on any musical instrument, may vary either in pitch, loudness, or in quality. The range of any individual voice is generally about two octaves. The pitch of the voice usually employed is determined chiefly by the length and mass of the vocal cords. Thus in children the voice is high-pitched. Before and at puberty there is a considerable development in the size of the larynx in both sexes. This is especially marked in the male, and accounts for the sudden drop in pitch ('breaking') of the voice. In the female the greater size of the larynx is chiefly perceptible in the increase in fulness and richness of the voice which occurs at this age. The ordinary human range, from deepest bass to highest soprano, is about four octaves. In singing, the voice may be produced in various ways, *i.e.* in different registers. Thus we distinguish the chest register, the middle register and the head register. The deeper notes of any individual voice are always produced in the chest register. Observation of the vocal cord shows that when producing such notes the glottis forms an elongated slit, all the muscles which close the glottis and increase the tension of the cords being in action. The vocal cords are relatively thick and broad and can be seen to vibrate over their whole extent. When singing with the head voice, the vibrations of the cord are apparently confined to their inner margins; the aperture of the glottis is wider in front than behind, so that more air escapes during phonation by this method than in the production of the chest voice.

In order to change the pitch of the note the following means are probably employed in the larynx:

(1) Alteration in the tension of the vocal cords.

(2) Alteration in the length of the part of the vocal cords that is free to vibrate, which can be accomplished by the approximation of the arytenoid cartilages to one another, or by their approximation to the thyroid cartilage.

(3) The alteration in the shape of the vocal cords, which is determined by the activity of the different portions of the internal thyro-arytenoid muscles.

(4) The varying pressure of the blast of air passing through the glottis.

The loudness of the tone produced is practically proportional to the force of the blast of air employed. The quality or timbre of the voice depends not so much on the vocal cords as on the accessory resonating

apparatus, represented by the trachea and chest and by the cavities of the mouth and nose. The greater part of the education involved in voice training is directed to the modification of the shape of the mouth cavity, so as to secure the greatest possible fulness, *i.e.* richness in overtones, of the tone produced in the larynx.

THE ACOUSTICS OF SPEECH

The sounds employed in speech, viz. vowels and consonants, are produced by modifying the laryngeal tones by changes in the shape of the throat, mouth and nasal cavities. Vowel sounds are continuous, whereas the consonants are produced by interruptions, more or less complete, of the outflowing air in different situations. In whispering speech there is no phonation at all, but the sound is produced by the issue of a blast of air through a narrow opening between the lips, between the tongue and soft palate, or between the tongue and the teeth.

VOWELS. During phonation the expired air is forced under pressure between the tense vocal cords thus setting them into vibration. A series of fairly regular puffs of air thus pass up the throat to the mouth and nose. This series of puffs constitutes a musical tone which is very rich in harmonics. Certain of these harmonics may be emphasised by the resonant vibrations of the throat, mouth or nose. The various vowels are produced by the variations in the pitches of these accentuated harmonics. Thus on singing ū (as in tool) with a fundamental pitch of 100 v.p. sec the third harmonic (400 v.p. sec.) and the seventh harmonic (800 v.p. sec.) are accentuated. If, on the other hand, the third and the twenty-first harmonic be accentuated the vowel becomes, i, as in will.

The characteristic frequencies of components of the vowel sounds when the fundamental is 100 v.p. sec. are given in the table below.

Series	Speech Sound		Low Frequency	High Frequency
I.	ū	mule	**400**	800
	ō	mole	**500**	850
	au	maul	**600**	950
	ŏ	moll	**700**	1150
	ar	marle	**825**	1200
II.	ē	feat	375	**2400**
	i	fit	450	**2200**
	ā	fate	550	**2100**
	ă	fat	750	**1800**
III.	ō	foal	**500**	**800**
	ur or er	furl	**500**	**1500**
	ĕ	fell	**550**	**1900**
	ā	fail	**550**	**2100**

In the first series the low frequency resonance is loud and the high frequency one is quiet, and they rise in pitch together. In the second series the converse is found to be the case, for the low frequency resonance is quiet and the high frequency resonance is loud; moreover, as one rises, the other falls. In series III., which may be called the transitional series, since it

connects series I. and II., the low frequency resonance remains approximately constant in pitch, whereas the high frequency resonance changes greatly.

If these three series be plotted on a graph with the low frequencies as abscissæ and the high frequencies as ordinates a triangular figure is obtained.

There has been much controversy as to whether the pitch of these harmonic components changes at all with the pitch of the voice, or varies in different individuals. Some said that they did not change, others that their pitch kept in constant ratio with the pitch of the note sung; if the note doubled in pitch, so also did those of the vowel components. Several methods have been employed for investigating this point:

(1) By recording the vibrations emitted by the voice by means of the manometric flame.

(2) By analysis of the vibrations recorded by means of a gramophone.

(3) By measuring the intensity of vibration of series of resonators. All the above methods show that there must be some change, even if it is slight.

(4) By running a gramophone record of a bass voice at an increased speed so that the notes were those of a treble. If now the pitches of note and vowels were in constant ratio, the quality of the vowels should not change when the speed is thus increased. Experiment shows that the words are greatly altered, losing their O's and A's and taking E and I instead. This shows clearly that the change in pitch of the vowels is not nearly as great as that of the note sung with them. We must conclude therefore that neither those who say there is no change, nor those who say there is constant ratio, are right, but that the truth lies between the two extremes. Diphthongs are produced by changing the form of the mouth cavity from that of one vowel sound to another, thus AI (the English I) = ah-ee run together and abbreviated.

CONSONANTS. These are sounds produced by a sudden check being placed in the course of the expiratory blast of air by closure of some part of the pharynx or mouth. They are classified into labials, dentals, or gutturals, according as the check takes place at the lips, between teeth and tongue, or between back of tongue and soft palate. Each of these again can be divided into soft and hard consonants as they are accompanied or not by phonation. Thus when we pronounce D the production of the laryngeal sounds goes on during the check of the sound produced at the teeth, whereas with T there is an absolute interruption of phonation during the pronunciation of the consonant. It is thus practically impossible to make any marked difference between hard and soft consonants when whispering.

In the production of nasal sounds such as NG, the mechanism is the same as for the production of B, D, G, except that the posterior opening of the nares is not kept shut by the soft palate, so that part of the sound comes continually though the nasal passages, when it acquires a peculiar resonance. These sounds are on this account often spoken of as 'resonants.' The aspirates are produced by the passage of a simple blast of air through a narrow opening which may be at the throat as in H, between tongue and teeth as in TH, or between lips and teeth as in PH or F.

The vibratives, such as R, are formed by placing the tip of the tongue, or the uvula, or the lips, in the path of the blast of air so that they are set into vibration by the blast. The vibrative R employed in English is entirely due to the tongue.

The sibilants, which may be voiceless, as in S, or accompanied with

phonation, as in Z, consist of continuous noises produced by a narrowing of the path of the air between the tongue and the hard palate. They are therefore similar in production to the aspirates. In the production of the sound L, the tongue is applied by its edge to the alveolar process of the upper jaw, so that the air or voice escapes by two small apertures in the region of the first molar and between the inner side of the cheek and the teeth. The acoustic characters of these various consonants are still but imperfectly studied.

Much work has recently been done, notably at the Bell and H.M.V. laboratories, to determine the conditions necessary for speech to be intelligible. In the first place the sounds must have adequate intensity. In the second place an adequate band of frequencies must be present. Thus on cutting out low frequency tones from 16 to 400, no loss of intelligibility results. On cutting off from 16 to 500 a 2 per cent. loss results. On cutting out from 16 to 600 a 10 per cent. loss results. With regard to high frequencies, on removing those above 4000 to 5000, little or no loss is found. On cutting off those above 3000, there is a 10 per cent. loss in intelligibility. Thus for good speech, frequencies of 500 to 4000 should be transmitted to the ears. For adequate speech, probably 600 to 3000 would be sufficient. In the third place, " hang-over " or reverberation must not exceed about half a second. When a sound is generated in an empty, undraped hall, it continues for some time to reflect to and fro with gradually decreasing intensity. This phenomenon is called reverberation. Knudson carried out investigations in a room the reverberation of which could be altered by lining it with various thicknesses of felt. He found a decrease of intelligibility of speech of 7 per cent. for each second of reverberation time. A room with a reverberation time of half a second should cause a $3\frac{1}{2}$ per cent. decrease of intelligibility, which is a hardly noticeable amount.

THE NEUROLOGY OF SPEECH

The intricate act of speech involves the employment of three different mechanisms: (*a*) The lungs, (*b*) the larynx, and (*c*) the cavities of the pharynx, mouth and nose. The lungs develop the necessary air pressure. The vocal cords of the larynx set the air into vibration with the required pitch. The cavities above, *e.g.* the mouth, modify the intensity of the tone and the relative intensities of its overtones so as to produce words.

THE LUNGS. For purposes of respiration the lungs are alternately filled and emptied by the synchronous action of the muscles of the chest wall and of the diaphragm. In addition, the muscles of the abdomen and arms and neck may be called into play when occasion demands more thorough ventilation. These muscles are innervated by nerve fibres which originate in the cells of the anterior horn of grey matter in the spinal cord. These same muscles are synchronously brought into play for purposes of phonation by voluntary impulses which, for a time, replace the normal respiratory rhythm. In general, inspiration is quickened, the vocal cords are separated, and air pours into the lungs. The vocal cords are now brought together and put under tension. Expiration now begins, and air commences to pass between the cords; these vibrate, producing sounds which are modified into speech. Expiration is prolonged until we have completed the sentence. The expiratory pressure even in quiet speech is above that during ordinary respiration, while in shouting not only the ordinary expiratory muscles are forcefully contracted, but the accessory

muscles of expiration (*e.g.* the abdominal) are freely made use of as well. Considerable muscular effort is required for loud speech or song. So great may it be that the individual becomes breathless as he does when performing other kinds of severe muscular effort.

THE MUSCLES OF THE MOUTH, &c. There are six points at least at which modification in diameter of the air cavities above the vocal cords may be produced: (*a*) The false cords, innervated by the recurrent laryngeal branch of the vagus nerve; (*b*) the pharynx at the level of the root of the tongue, by the pharyngeal branch of the vagus; (*c*) the buccal cavity near its posterior part, by the elevation or depression of the soft palate, supplied by the spinal accessory nerve; (*d*) the buccal cavity near its middle, by the elevation or depression of the dorsum of the tongue, innervated by the hypoglossal nerve; (*e*) the buccal cavity near its front, by the approach of the tip of the tongue to the upper teeth; (*f*) the buccal cavity at its anterior outlet, by the approximation of the lips (facial nerve).

In addition, the mouth cavity may be varied in size by the change in angle of upper and lower jaw (trigeminal nerve), and the nasal cavity may be allowed to communicate with the pharynx at its posterior outlet (spinal accessory nerve).

We may summarise these nervous controls by saying that the seventh, tenth and twelfth cranial nerves play the principal part in controlling this mechanism of speech.

THE CORTICAL CENTRES OF SPEECH. At one time it was thought that there was a circumscribed motor area for speech. It was called Broca's area after its discoverer, and occupies the posterior part of the inferior frontal gyrus. In right-handed people it is found on the left side of the brain only, and *vice versâ.* Destruction of this area undoubtedly causes dumbness, but histological evidence shows that it is an association area only. After destruction of this area the lungs can still be voluntarily ventilated. The cords can still be approximated and set into vibration, the false cords, pharynx, tongue, and lips can all be separately moved, but the complex correlation of all these separate motor functions is no longer possible. We may say, then, that, as the result of external stimuli, impulses reach Broca's association area, and by it they are distributed in suitable combination to the motor cortex of lips, tongue, cords, diaphragm, &c. The impulses travel down the axons of the Betz cells thus innervated, through the corona radiata, internal capsule, mid-brain, and pons, to reach either directly or through intermediate connections the motor nuclei of the seventh, tenth, and twelfth cranial nerves, and the anterior horn cells of the spinal segments involved in the act.

Broca's area is by no means the only important cortical association area involved in speech; others are found near both the auditory and visual cortex.

APHASIA. It has been usual to divide the disorders of speech, known as aphasia, into the following groups: (1) motor aphasia of Broca; (2) sensory aphasia of Wernicke; (3) alexia; (4) anarthria. A more fundamental idea of the processes involved will, however, be obtained if it is remembered that there are two ways in which the necessary ingoing impressions are received. namely, by reading written or printed words and by hearing spoken words, There are, correspondingly, two different mechanisms by which language may be conveyed, namely, by writing and by speaking. Consideration of these facts would suggest that there may be eight different possible defects: (1) vision may be perfect and yet there may be complete inability in understanding written or printed words (alexia); (2) hearing may be perfect and

yet the man may be incapable of understanding what is said to him (this is called sensory aphasia of Wernicke); (3) he may be able to use his hands and to draw with a pencil and yet be unable to write (agraphia); (4) he may be able to use his lips and to make noises with his throat and yet be unable to speak (anarthria or motor aphasia of Broca). Each of the above defects concerns one particular mechanism alone. Other possible defects may concern two mechanisms, one ingoing and one outgoing. Thus (5) there may be ability to read with inability to copy what is read, or (6) ability to read but inability to read aloud, or (7) ability to understand what is spoken but inability to write it down to dictation, or (8) ability to understand what is spoken and yet inability to repeat it.

The neurological basis of language must be regarded as co-extensive with the sensory centres, and with the whole region of lower association. We might speak of auditory and visual word-centres as located in the visuo-psychic and auditory psychic centres. There is probably, however, no word, still less a collection of words, expressing an idea which does not involve the activity of practically all parts of the cerebral cortex. As Bolton points out, "a word, such as 'mouse,' at once sets in effect processes of association which pass to every projection sphere with the solitary exception of the gustatory, and even this may be aroused in a person who has eaten a fried mouse in the hope of thereby recovering from an attack of whooping-cough." The amount of impairment of intelligence will vary in different cases according to the extent of the lesion. Thus, softening affecting the occipital lobe may, with hemianopia, cause 'word-blindness' or 'alexia,' a loss of power of appreciating the meaning of written words. In most individuals, and certainly in the uneducated, this power may be cut out altogether without interfering considerably with the mental powers. On the other hand, from babyhood upwards we have learnt the meaning of words and their grouping by auditory impressions. If the whole of the auditory associations be destroyed by an extensive lesion in the first and second temporal convolutions, the resulting loss of word appreciation, sensory aphasia, will be attended with great diminution of mental powers. It must be remembered that the area of Wernicke is not a sensory centre, but a centre of association between the various sense impressions, especially those of hearing and sight. It may therefore be spoken of as an intellectual centre. Pure motor aphasia, if it exists, is always anarthria and is due to a lesion in the lenticular zone, *i.e.* in the lenticular nucleus and its neighbourhood, in the anterior part and the genu of the internal capsule and possibly in the external capsule.

THE FUNCTION OF EXPRESSION. Although, since it is extremely exact, we rightly give precedence to speech as a means of conveying our ideas to one another, other modes of expression are of considerable interest since they probably once played a more prominent part than they do at the present day. No attempt will be made here to analyse them in detail. They may be divided anatomically into facial, manual, corporal, and pedal. The first comprises all those movements of expression of the superficial muscles of the face, the lips, the jaw, tongue, &c., which are called into play. Thus, corrugation of the superficial muscles of the forehead indicates surprise or worry; if the eyebrows are raised at the same time it indicates surprise; if they are lowered it indicates anger. The orbicularis muscles of the eyes when relaxed show astonishment or interest, and when contracted indicate boredom. Tremor of the lids expresses extreme mental agitation. The lips may be pursed up to show doubt, spread out in a smile, or separated in a grin.

Manual expressions are not so easily analysed as the above, but may be divided arbitrarily under a time, space, and intensity distribu-

tion. For example, the placing of the first finger on the lips to give the order for silence; patting the hands quietly together to signify approval; the slow raising and lowering of the right hand, palm downwards, to signify go slowly or stop. They are largely matters of convention, and some races use them much more than others. The conductor of an orchestra will show many examples of the expressions which come under this category.

As examples of corporal expressions, we have the leaning forward in a chair when interested, or leaning back when bored, or crouching as if to spring when in disagreement with what is being said, or when angered with the speaker, and as instances of pedal expressions, the shuffling of the feet when the mind is anxious, or the stamping of the foot when angry.

All the above are under the control of the will. In addition, there are the emotional symptoms controlled by the sympathetic system which are not under voluntary control—namely, the erection of the hair (the hair 'stands on end'), the dilatation of the pupil, the activity of the sweat glands, stimulation of the lachrymal glands expressing sorrow, and the cessation of the secretion of the buccal glands when the mouth becomes dry accompanying an anxious state of mind.

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CHAPTER XXVII

CUTANEOUS SENSATIONS

THE skin, being the outermost layer of the body, represents the tissue or organ by which the organism is brought into relationship with its environment. In the widest sense of the term the skin is protective. This function it discharges by virtue not only of its physical properties, but also of its rich endowment with sense organs, by means of which the intracorporeal events can be correlated with those occurring outside and immediately affecting the organism.

We are accustomed to distinguish several qualities of sensation among those having their origin in the skin, the chief of which are the sense of touch, including that of discrimination, the sense of pain and the sense of temperature. The very different qualities of sensation included under these three classes suggest that there may be a special mechanism, or class of mechanism, for each sense, and a careful investigation of the sensory qualities of the skin surface bears out this idea. Isolated stimulation of minute areas on the skin does not excite all the sensations together, but only a sense of touch or of pain, or a sense of cold or warmth. We are therefore justified in dealing with each of these sensations separately.

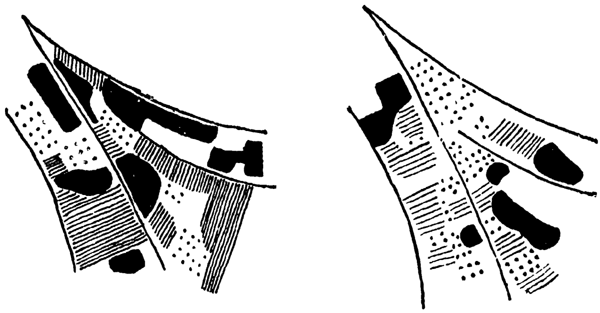
THE TEMPERATURE SENSE

By means of the skin we can appreciate when a body coming in contact with it is either cold or warm. If the body is at the same temperature as the skin, as a rule no sensation of temperature is excited. It was formerly thought that the sensations both of heat and cold were determined by the excitation of one and the same end organ. Warming of this end organ would produce a sensation of warmth, while a diminution of its temperature would produce the sensation of cold. Careful investigations of the distribution of the temperature sense by Blix and Donaldson has shown that this opinion cannot be maintained. If a small surface warmed to a few degrees above the temperature of the skin be moved over any part of the surface of the body, *e.g.* the back of the hand, it is found that the warmth of the instrument is not equally appreciable at all parts of the surface of the skin. At some points the sensation of warmth will be very pronounced, but between these points the sensation of warmth may be entirely wanting and the instrument may be judged to be of the same temperature as the hand itself. In this way a series of 'warm points' may be mapped out. On now cooling the instrument a few degrees below the temperature of the surface of the body and then moving it over the surface in the same way, it will be found again that the coolness of the instrument is appreciated only at certain points; these can be regarded as 'cold points' and as containing the nerve endings by the excitation of which the sensation of cold is produced. If the warm points be pricked out in red ink and the cold points in blue ink, it will be seen that they do not in any way correspond.

A convenient instrument for this purpose is the one invented by Miescher, consisting of two tubes cemented together and communicating at a small flattened extremity,

which is applied to the surface of the skin; through the tubes water can be led at any desired temperature, which is read off by a thermometer placed within the tube. Having mapped out the warm spots, it may be shown that they are excitable by means of mechanical or electrical stimuli and that the sensation produced is the same as if they had been excited by their adequate stimulus, viz. a rise of temperature.

Exact Location of the spots is rendered difficult by the irradiation of the sensation produced, so that it is difficult to refer the sensation of warmth or cold definitely to the point stimulated. An investigation of the topography of these warm and cold spots shows that the apparatus for the appreciation of cold is much more extensively distributed over the body than that for the appreciation of warmth, as is evidenced from the diagram (Fig. 301) giving the topographic distribution of the cold and warm sense organs on the palm of the hand. Waterston (1923) claims that in the case of both heat and cold, the topographic distribution of the spots alters continually. In skin suffering from a mild erythema, all parts respond to warmth, that is, all signs of a punctate arrangement have gone. He concludes that all parts of the skin can respond to both heat and cold, the punctate



Cold spots. Heat spots.

Fig. 301. Heat and Cold Spots on part of Palm of right Hand.

The sensitive spots are shaded, the black being more sensitive than the lined, and these than the dotted parts. The unshaded areas correspond to those parts where no special sensation was evoked. (Goldscheider.)

arrangements found at a single examination being due to some parts being more active than others. The skin capillaries are stated to show a similar change from time to time in the quantity of blood which they transmit. The temperature sense is best marked in the following regions of the body: the nipples, chest, nose, the anterior surface of the upper arm and the anterior surface of the fore-arm, and the surface of the abdomen. It is much less marked on the exposed parts of the body, such as the face and hands, and is but slight in the mucous membranes. Thus it is possible to drink hot fluid, such as tea, at a temperature which would be painful to the hand, and still more to any other part of the body. The scalp is also very insensitive to changes of temperature. The acuteness of the temperature sense varies considerably with the condition of the skin and with the previous stimulation of the sense organs. The sense is most acute at about ordinary skin temperature, *i.e.* between 27° and 32° C. At this temperature the skin can appreciate a difference of $\frac{1}{5}$° C. When the skin is very cold or very hot, the temperature sense is not nearly so delicate. This sense presents the phenomenon of adaptation in a marked degree. It is a familiar experience that, on coming from the external air on a cold day into a warm room, a sensation of warmth is experienced all over the body.

In a few minutes this sensation wears off. On now leaving the room to go outside again, the sensation of cold is at once appreciated, to disappear in its turn after a few minutes. The effect of adaptation is still better shown by the experiment of taking three basins of water, *a*, *b*, and *c* ; *a* contains cold water, *b* tepid water, *c* hot water. The left hand is immersed in the cold water and the right hand in the hot water for a few minutes. On now placing both hands into the basin of tepid water, it feels hot to the left hand and cold to the right hand. Such experiences as this led Weber to the conclusion that the essential stimulus for the temperature sense was not the actual temperature to which the sense organs were subjected, but the fact of a change of temperature. He imagined that, while the temperature sense organs were being warmed, a sensation of warmth was produced, and when their temperature was being lowered, a sensation of cold. Such a theory, however, would not account for the fact that, above a certain temperature, water may feel warm and the feeling may continue so long as the skin continues to be stimulated. On a cold day the air may feel cold to the face and the feeling may last the whole time that the face is exposed. Moreover we have in the temperature sense, conditions which remind one of the after images which occur in the eye. If a cold object be pressed on the forehead and then removed, the sensation of cold lasts some little time after the object has been removed. In this case a sensation of cold is produced although the end organs are being gradually warmed up after the removal of the cool object. In order to account for these facts Hering, at a time when the differentiation of hot and cold spots had not yet been effected, suggested that the temperature sense organs could be regarded as having a zero point at which no sensation was produced. If their temperature was raised above this point, a sensation of warmth was produced, and *vice versâ*. The zero point, however, was not a fixed one, but could move upwards to a certain extent on prolonged exposure to high temperature, or downwards on prolonged exposure to a low temperature. In the light of the researches of Blix and Goldscheider, we should have to apply Hering's theory of a zero point to each of the temperature end organs separately.

A cold pencil passed over a warm spot evokes no sensation whatsoever. If, however, a pencil considerably warmer than the skin be passed over a cold spot, this may be excited so that the paradoxical result is produced of a sensation of cold as the result of stimulation by a warm body. It is a familiar fact that the immediate effect of entering a hot bath is very much the same as that of entering a cold bath, viz. a rise of blood pressure and contraction of the unstriated muscles of the skin and hair follicles with the production of 'goose skin.' It has been suggested that the distinctive quality of a sensation of *hot* as compared with that of *warm* is due to the simultaneous stimulation of warm spots and cold spots. With regard to the identity of the sense organs for warmth and cold, J. P. Nafe's theory must be referred to, namely that the sensation of warmth is brought about by dilatation of the skin vessels, and that to some extent the sensation of cold is brought about by their contraction. He supports his theory by evidence among which we may notice (1) the sensation of flashes of warmth and cold when the arteries of the skin are punctured * ; (2) the striking proximity of blood vessels to cold points ; (3) the absence of blood vessels and the absence of cold and warmth sensations from the cornea ; (4) the similarity of the reaction times for the appreciation of warmth and cold, and the contraction and dilatation of the skin blood vessels.†

* BAZET and McGLONE. *Brain*, **51**, p. 18, 1928.
† See p. 1057, "Handbook of General Expt. Psychology" Clark Univ. Press, 1934.

THE SENSE OF TOUCH

By means of the sense of touch we arrive at a conclusion as to the qualities, such as shape, texture, hardness, &c. of the bodies with which the skin is in contact. In this judgment, however, very many other sensations are involved besides those which can be regarded as strictly tactile. Thus, the hardness of an object signifies its resistance to deformation, besides its power of deforming the skin surface with which it is in contact; the former quality, *i.e.* of resistance, is one which involves the muscular sense, since we judge of it by the extent to which we can move our muscles without causing any alteration of the surface of the object.

The tactile sensibility of the skin as a whole, like its temperature sensibility, was thought to have a punctate distribution. Recent work on the subject, by Waterston (1923), throws very great doubt on the validity of this idea.

RESPONSE TO DIFFERENT STIMULI. The adequate stimulus for the tactile nerve endings is not so much pressure, as deformation of surface. It appears to matter little whether the surface be deformed by pulling it or by pushing an instrument into it. The ineffectiveness of mere pressure is shown by dipping the finger into a vessel of mercury. The sensation of pressure is noted only at the point where the finger passes through the surface

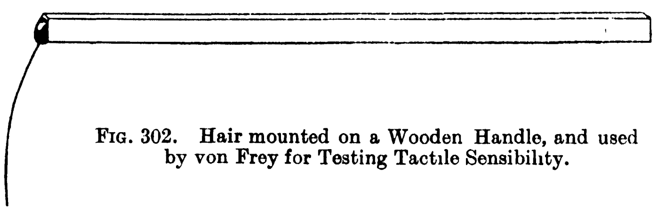


FIG. 302. Hair mounted on a Wooden Handle, and used by von Frey for Testing Tactile Sensibility.

of the mercury, and this is the only part where there is an actual deformation of the skin, due to the sudden passage from the pressure of the mercury to the negligible pressure of the outside air. The tactile apparatus is smarter in its response than any other of the sense organs. On this account stimuli are still perceived as discrete, when they are repeated at a rhythm which would result in complete fusion in the case of any of the other sense organs. Thus if a bristle be attached to a tuning fork and allowed to press on the skin, the vibrations of the fork are perceived by the ear as a continuous sound and by the skin as a series of discontinuous taps. Faradic currents when applied to the skin can be perceived as separate when repeated at the rate of 130 per second. The sensations evoked by placing the finger against the edge of a cog-wheel do not become continuous until the wheel is revolving at such a rate that the stimulation on the skin by the serrations occurs at a greater rate than 500 or 600 per second. The tactile apparatus resembles all the other skin sense organs in showing adaptation. A stimulus after continuing for some time may become ineffective. We are usually entirely unaware of the stimulation of our skin by the pressure of the clothes, and even an unwonted stimulation, such as that of the mucous membrane of the mouth by a plate carrying artificial teeth, though almost unbearable during the first day, rapidly becomes less, and in a few days it is not perceived at all.

In order to test the sensitiveness of touch we may use the method introduced by Hensen, viz. the bending of a glass-wool fibre. We can

determine the pressure at which any given fibre will bend, and if we find by trial the fibre which just evokes sensation when pressed on the skin, we know exactly the force which we are applying to the skin. Von Frey employed hairs of different thickness for the same purpose (Fig. 302). The following represents the minimal excitability of the surface of different parts of the body when tested in this way:

	Grm per sq. mm.
Tongue and nose	2
Lips	2·5
Finger-tip and forehead	3
Back of finger	5
Palm, arm, thigh	7
Fore-arm	8
Back of hand	12
Calf, shoulder	16
Abdomen	26
Outside of thigh	26
Shin and sole	28
Back of fore-arm	33
Loins	48

The sensitiveness of the sense organs in the skin is probably much greater than that of the nerve trunks themselves. Thus Tigerstedt found that the minimal mechanical stimulus necessary to excite the exposed nerve amounted to 0·2 grm. moving at 140 mm. per second. For the touch spots, von Frey found that 0·2 grm. moving at 0·17 mm. a second is an adequate stimulus.

In testing the sensibility of any surface, it is important to remember that the hairs themselves form very effective tactile organs. The touch spots are distributed in greatest profusion around hair follicles, and there is a rich plexus of nerve fibres round the root of each hair. A slight touch applied to the hair acts on these as on the long end of a lever, the hair being pivoted at the surface of the skin, so that pressure on the hair, increased five or more times in force, is transmitted to the hair follicle and the surrounding nerve endings. The actual sensibility of any part is therefore much diminished by removal of the hairs. On 9 sq. mm. of the skin, from which the hairs had been shaved, the minimal stimulus necessary to evoke a tactile sensation was found to be 36 mg., whereas on the same surface before it was shaved 2 mg. was effective.

WEBER'S LAW. The smallest increment or decrement of stimulus which determines a perceptible difference of sensation must, according to Weber's law, always bear the same ratio to the whole stimulus. In measuring such differences it is best to apply the stimulus successively to the same surface of the skin, rather than simultaneously to adjoining areas. The time interval between two successive stimuli should not be more than five seconds and the duration of the stimuli should be equal. Weber found that in the terminal phalanx of the finger the minimal perceptible difference was about one-thirtieth, but the ratio was not the same for all regions of the skin nor for all individuals. The following represents the liminal difference in various skin regions:—

Forehead, lips and cheeks	1/30th to 1/40th
Back of fore-arm, of leg and of thigh; back of hand, and first and second phalanx of finger, &c.	1/10th to 1/20th
All parts of the foot, leg and thigh	more than 1/10th

THE SPATIAL QUALITY OF TOUCH. DISCRIMINATION. If any part of the skin be stimulated, the subject of the experiment can tell at once the exact situation of the excited spot. If two points be stimulated simultaneously, excitation is perceived as double, *i.e.* as proceeding from two points, provided the distance between the points exceeds a certain amount, varying in different parts of the body. The power of discrimination, *i.e.* of judging whether a stimulus is single or double, can be tested by arming the points of a pair of compasses with small pieces of cork and then seeing how far apart the points must be when pressed on the skin in order that the stimulus may be perceived as double. The following Table represents this distance for various regions of the body :—

DISTANCE IN MM.

Skin region	mm
Tip of tongue	1·1
Volar surface of finger tip	2·3
Dorsum of third phalanx	6·8
Palm of hand	11·3
Back of hand	31 6
Back of neck	54 0
Middle of back, upper arm, and thigh	67·1

When touch spots are sought out for stimulation with the points of a compass, the distance at which the excitation is perceived as double is much diminished, as is shown by the following Table of distances for the touch spots in millimetres :

Skin region.	Distance of touch spots
Volar side of finger tips	0 1
Palm of hand	0 1
Fore-arm (flexor side)	0 5
Upper arm	0 6
Back	0 4

The compass points are perceived to lie apart with a special distinctness when they are applied to touch spots lying on different lines which radiate from the hair follicles. The figures given in the first Table have no relation to touch spots, but show the average distance over which an excitation can be perceived as double.

The delicacy of discrimination of any part is largely associated with its mobility. Thus, in the arm the delicacy increases continuously from the shoulder to the finger tip. If the localising power for touch on the shoulder be taken as 100, that of the finger tips will be represented by 2582. In the same way there is a continuous decrease of the distances of discrimination as we pass along the cheek from the ear to the lip, *i.e.* from the non-mobile to the mobile part. The power of discrimination is increased to a certain extent by practice and largely diminished by fatigue. Any factor which diminishes the tactile sensibility of the part, such as cold, will also diminish the power of discrimination.

LOCALISATION OF TOUCH. The fact that we can localise the point of stimulation shows that every tactile sensation derived from the surface of the body, besides the qualities of intensity and extensity, has also associated with it a characteristic quality dependent on its position. This localised quality of a tactile sensation was called by Lotze 'local sign.' Among psychologists there has been much discussion as to how far this local sign is an inborn attribute of the sensation of every point on the body surface, or how far it is acquired by experience and based on memory of movements

and muscular impressions. In the retina we have a sense organ which, like the skin, possesses local sign but in far higher degree, the power of discrimination of the retina being three thousand times as great as that of the most sensitive part of the skin. Cases of congenital cataract occur in which the subjects have been blind from birth. By extraction of the cataract we can give such persons the power of sight. It is found that at first there is no power of localising visual impressions. The local sign is developed only in response to experience, by comparing simultaneous visual, tactile and motor sensations. By analogy we might ascribe the local sign of cutaneous sensations to a similar causation. Our study of the spinal animal has indeed given us a physical or histological conception of local sign. We know that stimulation of any part of the body evokes an appropriate reaction, the nature of which is determined by the central connections of the entering nerve fibres. A fibre entering at one segment must therefore come into relation with a different set of motor cells from those which are set into action by a fibre entering one segment lower down. Every nerve fibre from the skin will therefore have an appropriate complex of motor paths in functional connection with its central endings ; and when the activity of these reflex paths comes to be represented in consciousness, it is evident that the sensation derived from any one point must differ from that derived from any other point of the skin, by virtue of the differing motor events actually or potentially excited from the two points. In ascribing local sign to coincident muscular sensations and to the memory and experience of past movements, we are therefore giving but an imperfect explanation; since the difference between the sensations from different parts, which is at the bottom of our powers of localisation, has its origin in the structure of the central nervous system itself and is present from the very beginning of the evolution of a reactive nervous system.

PROJECTION OF TOUCH. Since the alterations in the surface of the skin which give rise to tactile sensations are habitually caused by contact with external objects, we come to regard the sensations themselves, not as changes in the skin, but as qualities of the object which touch the skin, *i.e.* we project the sensation. The projection is, however, not so great as in the case of visual sensations. Cutaneous sensations we always consider as qualities of an object immediately affecting and altering the condition of ourselves, whereas the visual sensations are referred at once to objects lying right away from ourselves, so that we are not aware that any change has taken place in our bodies as a result of the entering of rays of light into the eye.

It is remarkable to what extent projection of touch sensation may occur. Thus a surgeon actually lengthens his fingers by using a probe. When he is probing for dead bone he feels the grating of the bone, not at his finger tips, but he projects the sensation to the end of the probe. In the same way tactile sensations evoked by the contact of bodies with the insentient endings of hairs are referred to the ends of the hairs rather than to the hair follicles where the nerve impulses actually come into being.

The dependence of local sign on habitual experience is shown by the various tactile illusions, such as the well-known experiment of Aristotle. If with the eyes shut we cross the first and middle fingers and bring them in this position in contact with a pea, we should at once say that two peas lay under the fingers. This is especially marked if the pea be rolled between the fingers. The two sides of the fingers which come in contact with the

pea usually touch two different objects, and these parts of the skin would have to be re-educated, *i.e.* their local sign would have to be changed in accordance with the changed conditions, before the pea would be perceived in its true state as single.

THE PAIN SENSE

When the pressure of a hard object on the skin is increased beyond that necessary to evoke a tactile sensation, at a certain pressure the quality of sensation changes and it becomes painful. For the evolution of the race as well as for the preservation of the individual this pain sense is all-important; it is the expression in consciousness of the reflexes of self-preservation which can be evoked in the spinal animal by stimuli which are nocuous, *i.e.* calculated to do actual damage to the tissues of the body. Thus when a sharp point is pressed on the skin, the sensation becomes painful just before the pressure is sufficient to cause penetration. The so-called trophic lesions, which occur in parts devoid of sensation, are determined for the most part by the lack of the pain sense and the consequent failure of the preservative reflexes of the part. It is remarkable that pain may result from changes in organs which are devoid of ordinary sensibility. Thus the intestine may be cut, sewn or handled without arousing any sensation whatsoever. A strong contraction of the muscular wall or increased distension of the gut will, however, evoke a griping pain. In the same way the ureters, which are normally devoid of sensation, can give rise to excruciating agony when they are contracted firmly on a retained calculus.

We are accustomed to distinguish many different qualities of pain, but on analysis it will be found that these qualities depend on the nature of the sense organ which is simultaneously stimulated. Thus a burning pain denotes simultaneous stimulation of the pain sense and of the nerve endings to the warm spots. A throbbing pain results when the vessels of the part are dilated and the part is tense with effused lymph, so that each pulse of the vessels causes an exacerbation of the painful stimulation and perhaps also stimulation of the tactile end organs.

The sense of pain has often been ascribed to over-maximal stimulation of any form of sensory nerve. Although it is true that over-stimulation of the auditory or optic nerve by a loud sound or a bright light may be extremely unpleasant, the sensations evoked do not partake of the characters of painful sensations such as would be produced by pricking or burning the skin. Moreover a careful investigation of the sensory points on the skin brings out the fact that there are, besides the tactile and temperature spots, other spots from which only painful sensations can be evoked. We have seen already that over-stimulation of a touch spot does not, as a matter of fact, cause pain. The pain spots which are distributed among the touch and temperature spots are insensitive to a low grade of stimulus. As the strength of the stimulus is increased, a point is suddenly reached at which the sensation evoked is painful. Moreover, in parts of the body, tactile and temperature sense are entirely wanting, though painful impressions can be easily evoked. The best example of this is seen in the cornea, minimal stimulation of which evokes pain, but nothing which can be regarded as a tactile sensation. The specific quality of pain sensation is shown moreover by the fact that in many cases of disease the sense of pain may be abolished without the sense of touch. Such a patient is said to suffer

from analgesia, but not from anæsthesia. When pricked on an analgesic part the patient can say that he is pricked, but has no objection to any amount of repetition of the stimulus, since the sensation is entirely devoid of painful character. In the case of the skin, pain is found to differ somewhat from the other skin sensations in being much more uniformly distributed, more difficult to locate accurately, and more hardy. Thus while most sense organs are rendered less sensitive by cutting off blood supply, pain at first reacts more violently.

Protopathic and Epicritic Sensations. As the result of experiments by Head and Rivers, in which a sensory nerve (the radial) in the arm was cut, and the returning sensibility followed during regeneration, it was thought that the sensations could be divided into three groups—epicritic, protopathic and deep. Deep sensations remained after the nerve had been cut, and consisted of pressure sense and pressure pain. Protopathic sensibility was the first to return and consisted of a vague pain sense and a sensation to hot objects

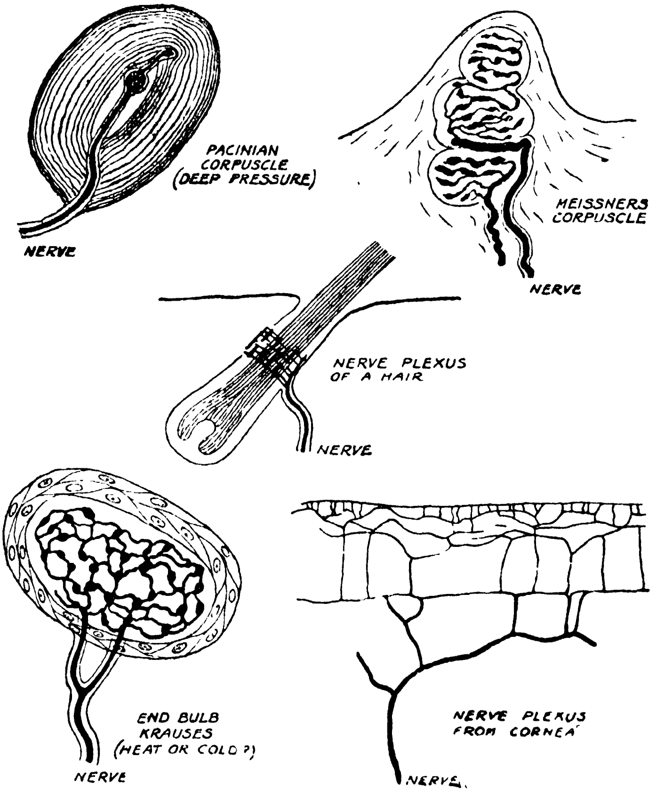


FIG. 303. Skin End Organs and the Sensations which they arouse. It has been supposed that corpuscles of the type of Meissner's corpuscle subserve pain sense, and that nerve plexuses, such as that illustrated for the cornea, are for touch.

above 38° C. and cold ones below 24° C. Epicritic sensation, the last to return, gave critical discrimination and localisation and differentiation of temperatures between 25° C. and 37° C.

But recent workers, Trotter and Davies (1909), and Boring (1915), who have repeated Head's and Rivers' experiment, and Carr (1916), who subjected Head's and Rivers' own data to analysis, agree in the conclusion that the distinction into protopathic and epicritic is purely artificial and must be finally abandoned.

THE HISTOLOGICAL CHARACTER OF THE ELEMENTS INVOLVED IN CUTANEOUS SENSATIONS

A very large number of different forms of sensory nerve endings have been described in relation to the skin. Their exact allocation among the different cutaneous senses presents considerable difficulties.

As regards touch, two kinds of elements are probably involved. In the first place, the most sensitive tactile apparatus are the follicles of the short hairs. Around these follicles we find a sheaf of nerve fibres, some of which end in the hair papillæ and others form a ring near the level of the openings of the sebaceous glands. The other tactile end organ is, according to Waterston (1932), the nerve plexus which terminates in the epithelial cells of the more superficial layers of the epidermis. The evidence he advances for this view is that on cutting away the superficial layers with a razor the sensation of touch is impaired or removed, leaving the sensation of pain unaffected. Other end organs, which are supposed to be stimulated by changes of pressure and therefore to be tactile, are the organs of Ruffini which occur in the papillæ of the palm and fingers and, lying more deeply, the elastic tissue spindles as well as the Golgi corpuscles and the Pacinian corpuscles in the subcutaneous tissue.

As regards pain, we know that in the cornea, which possesses only the pain sense, the sensory nerve endings are in the form of branches of axis cylinders among the epithelial cells. Since by a section of the skin parallel with the surface the touch spots can be removed, leaving the pain spots, Waterston concludes that the sensory end organs for pain are more deeply placed than those for touch, and he identifies pain with Meissner corpuscles. It would be possible, however, to explain Waterston's experiment on the basis of the usual assumptions (*i.e.* that pain originates in free nerve terminals and touch in Meissner corpuscles), by supposing that the sensation of pain which remains is due to irritation of the cut ends of the sensory nerves and not to stimulation of the Meissner corpuscles. Further experiments are required to clear up these points. We have at present no evidence as to the histological character of the organs by which the sensations of heat and cold are aroused. They are believed to be Krause's and Culls.

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CHAPTER XXVIII

SENSATIONS OF SMELL AND TASTE

EVERY living organism shows a susceptibility, *i.e.* a power of reaction, to chemical stimuli. These movements of attraction and repulsion are spoken of as positive and negative chemiotaxis respectively. The aggregation of leucocytes round microbes or other foreign particles in the tissues is also determined by their chemiotactic sensibility. *Chemiotaxis*, then, represents the faculty by means of which these minute organisms are able to adapt themselves to chemical changes in their environment and to react to chemical substances diffusing towards them from a distance.

On the other hand, a chemical sensibility of the body surface, or part of it, furnishes the criterion by which particles are accepted and ingested as food or rejected as useless or harmful. Consciousness in this case would be of something in contact with and affecting some part of the organism itself. The sensation would not be projected further than the periphery of the body.

These two kinds of chemical sense—the projected and the surface sense—are found throughout almost all classes of the animal kingdom, and in the higher animals at least are known as the senses of smell and taste. The former sense in many animals attains a high degree of complexity and is prepotent in determining the behaviour of an animal in response to the changes in its surroundings. In the elasmobranch fishes the olfactory lobes form the greater part of the higher brain, and extirpation of them produces a loss of spontaneity and of delayed reactions similar to that which can be brought about in higher types by extirpation of the whole of the cerebral hemispheres.

The sense of taste, on the other hand, is used only for sampling the nature of substances taken into the mouth and determining their ingestion or rejection. It is therefore much simpler in its extent and more susceptible of analysis.

THE SENSE OF TASTE

The end organs which subserve the function of taste are represented by the *taste buds*. These are oval bodies (Fig. 304) embedded in the stratified epithelium, which occur scattered over the tongue, a few being also found on the hard palate, the anterior pillars of the fauces, the tonsils, the back of the pharynx, the larynx, and the inner surface of the cheek. On the tongue they are found chiefly in the grooves around the circumvallate papillæ of man, and in the grooves of the papillæ foliatæ of rabbits. A few are also present on many of the fungiform papillæ. They consist of medullary and cortical parts, the latter being composed of columnar or sustentacular cells, the former of thin fusiform cells, the taste cells proper. The nerve fibres concerned with taste end in arborisations among these taste cells. The peripheral end of the fusiform cell projects as a delicate process through the orifice of the taste bud, so that it can come in contact with the fluids contained in the cavity of the mouth. A sapid substance, to stimulate these organs, must be in solution; hence quinine in powder is almost tasteless, owing to its slight solubility.

DIFFERENTIATION OF TASTE. The number of classes of tastes is very limited. We distinguish four primitive taste sensations, viz. sweet, sour.

bitter and salt, some authors adding to these an alkaline taste and a metallic taste. Many substances owe their distinctive character when taken into the mouth to the fact that they stimulate not only the taste nerves but also the nerve endings of tactile sensation. Thus acids, when in weak solution, have an astringent character besides their sour taste, and if strong produce a burning sensation. The primitive taste sensations can affect one another if excited simultaneously. With weak stimulation one taste may practically annul another. Thus a dilute solution of sugar is rendered almost tasteless by the addition to it of a few grains of common salt. If the primitive taste sensations are more strongly excited we get a mixed sensation, in which the components can still be distinguished. Thus, adding sugar to lemon juice not only diminishes its acidity but produces a mixed sensation, the quality of which is pleasant and in which the components, sour and sweet, can be easily distinguished. We get no such fusing of sensations as in the eye, where a sensation of white light may result from stimulation of the retina by two complementary colours. Stimulation of one kind of taste organ heightens the sensibility of the other taste organs. Thus after the application of salt, distilled water may taste sweet. The sense of taste may be investigated in three ways : (1) By ascertaining the greatest dilution of a substance that will cause a definite taste, (2) by ascertaining how long a solution of a substance must be applied for a taste to be aroused, (3) by ascertaining the least difference between the strengths of two solutions of the same substance which taste different to the experimenter.

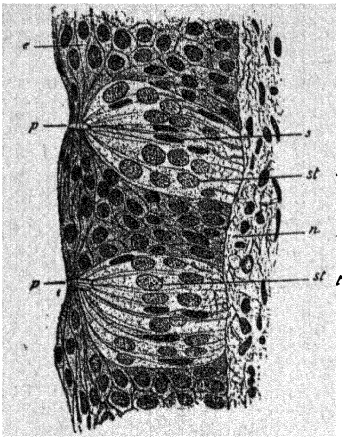

FIG. 304. Two Taste Buds from the Tongue. (KÖLLIKER.)

e. Stratified epithelium.
p. Opening or pore of taste bud.
n. Sub-epithelial tissue.
s. Gustatory cells.
st. Sustentacular cells.

By these methods we find :—

(*a*) The tongue is not equally sensitive at all points to all four tastes. Thus the back of the tongue is more sensitive to bitter, while the tip and sides of the tongue react more easily to sweet and sour substances. A difference may even be detected between the circumvallate papillæ themselves ; a mixture of quinine and sugar applied to one papilla may excite chiefly a bitter taste, while with an adjacent papilla a sweet taste may predominate.

(*b*) By certain drugs we can depress the sensibility of the taste organs, and we then find that the various tastes are affected to different degrees. Thus on painting the tongue with cocaine the first effect is a diminution of tactile and pain sensibility, so that the application of acid evokes a very sour taste without any of the astringent or stinging sensations normally aroused by the contact with the acid. After this point the taste sensations are also mostly abolished. The bitter sensation disappears first, then the sweet and then the sour, while the taste of salt appears to remain unaffected. On the other hand, if the leaves of *Gymnema sylvestre* be chewed, the sensations of bitter and sweet are abolished, leaving intact the acid and salt tastes, and also the general sensibility of the mucous membrane.

TASTE AND CHEMICAL CONSTITUTION. There is no doubt that the stimulating effect of any chemical substance on the taste nerves has relation

to its chemical constitution. Thus a sour taste is determined by the presence of H ions; the alkaline taste by that of OH ions. The fact that certain acids, *e.g.* acetic, have a stronger sour taste than would correspond to their dissociation, *i.e.* to the number of H ions present, is due to the fact that these acids penetrate more easily into the gustatory cells than the mineral acids with a larger dissociation co-efficient. All the *a*-amino-acids have a sweet taste. On the other hand, the polypeptides produced by the combination of these amino-acids, as well as the peptones derived from the hydrolysis of proteins, have a bitter taste. Most of the alcohols and sugars have a sweet

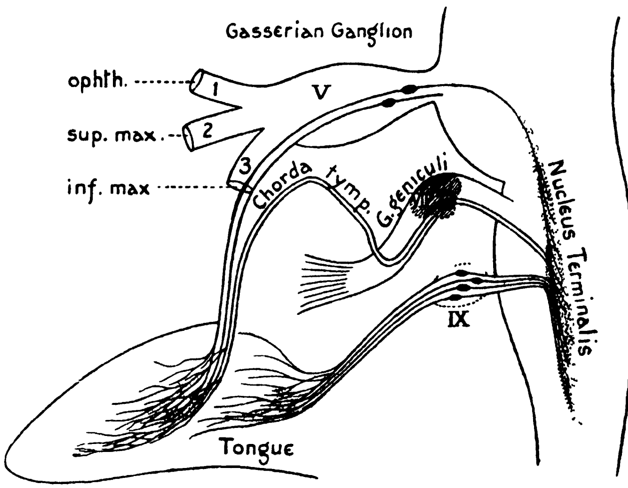

FIG. 305. Diagram showing Origin and Course of the Nerve Fibres of Taste.

taste, while the metallic derivatives of these substances are bitter. We do not yet understand the law which determines whether any given substance shall have a taste at all, and what its taste should be.

The nerves of taste are the glossopharyngeal, which supplies the back part of the tongue, and the lingual branch of the fifth nerve and the chorda tympani which supply the front part. All these fibres are probably connected with a continuous column of grey matter in the brain stem, which represents the splanchnic afferent nucleus of the fifth nerve, the nervus intermedius and the glossopharyngeal. Some authors have stated that all the taste fibres of the fifth nerve are derived from the glossopharyngeal by the communication through the tympanic plexus and the chorda tympani nerve, while Gowers has recorded a case of complete unilateral loss of taste in which there was a lesion destroying the fifth nerve, the glossopharyngeal being intact. It seems possible that the actual region of the taste nerve may vary, the fibres running to the splanchnic column of grey matter being contained sometimes in the fifth, sometimes in the glossopharyngeal, and sometimes in both.

Most of our so-called tastes should rather be designated flavours and are dependent not on the gustatory nerves but on the sense of smell. When the olfactory sense is destroyed, very little difference is to be perceived between an onion and an apple. The epicure with a fine palate has really educated his sense of smell and would be but little satisfied with the simple sensations derived from his tongue.

THE SENSE OF SMELL

The analysis of olfactory sensations is rendered difficult by the fact that this sense in man plays but a small part in his usual adaptations. We have thus to deal with a sense which is in many respects vestigial. We see traces of great complexity in its possibilities of performance, but are baffled in our endeavours to reduce the whole of the phenomena to the simpler factors of which they are composed. Moreover, like all vestigial functions, the extent to which the sense is developed varies from one individual to another. Many, for instance, are unable to appreciate the smell of vanilla, of hydrocyanic acid or of violets. On the other hand, in animals such as the dog, the olfactory sense seems to play a great part in determining behaviour, and the nervous associations, which are the physiological basis

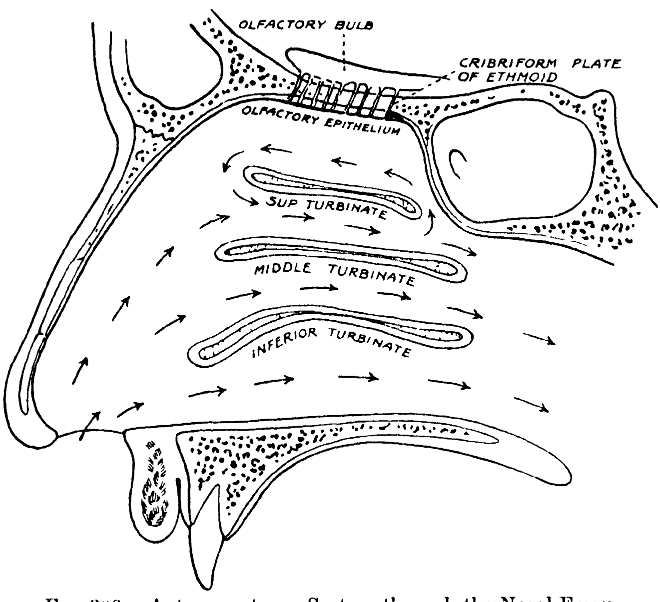

FIG. 306. Antero-posterior Section through the Nasal Fossæ.
The arrows show the direction of the air currents during inspiration.

of ideas, must in these animals be largely connected with olfactory impressions. Another factor which diminishes the importance of olfactory sensations in man is the ease with which the sense organ becomes fatigued. It often happens that the inmates of a room are perfectly comfortable and may perceive no fault in the ventilation, although a newcomer from the outside at once remarks that the air is foul.

THE ORGAN OF SMELL is situated at the upper part of the nasal cavities. Here the mucous membrane covering the superior and middle turbinate bones and the corresponding part of the septum is different from that covering the rest of the nasal passages. Over the lower parts of the nasal cavities the mucous membrane is of the ordinary respiratory type, and is composed of ciliated columnar epithelium containing a number of goblet cells. In the olfactory part the epithelium is much thicker, of a yellow colour, and apparently composed of a layer of columnar cells resting on several layers of nuclei. These nuclei belong to the olfactory cells proper, true spindle-shaped nerve cells with one process extending towards the mucus covering the free surface, while the other is continued along channels in the bone, and through the cribriform plate as one of the non-medullated olfactory nerve fibres. These nerve fibres enter the

olfactory lobes, where they terminate by a much-branched arborisation or end basket in the so-called olfactory glomeruli, in close connection with a similarly branched dendrite of the large *mitral* cells of the olfactory lobe. The axons from these latter carry the olfactory impulse towards the rest of the brain. In the connective tissue basis (dermis) of the mucous membrane are a number of small serous glands (Bowman's glands) whose office it is to keep the surface of the membrane constantly moist.

In ordinary respiration the stream of air never passes higher than the anterior inferior border of the superior turbinate bone, so that it does not come in contact with the olfactory mucous membrane. The sensations of smell which are aroused during ordinary respiration depend on diffusion from the respiratory air into the still air of the upper olfactory portion of the nasal cavity. The direction of olfactory attention is achieved by sniffing ; in this act the nostrils are dilated and the direction of the anterior part of the nasal respiratory chamber altered, so that the stream of entering air is directed towards the upper olfactory portion of the cavity.

The fact that the air, which enters the nasal cavity during respiration, does not come into direct relationship with the olfactory epithelium has the following advantages :—

(1) The cold inspired air does not come into contact with and cause damage to the sensory surface.

(2) Foreign particles carried by the air (including bacteria) do not get deposited there. The position of the epithelium at the very top of the nasal cavity is an additional safeguard.

(3) The olfactory epithelium is not dried by the rush of dry air across it.

(4) Noxious vapours only reach it indirectly and therefore do not cause permanent damage as they otherwise might.

The amount of substance necessary to excite sensation is extremely minute. Thus 0·01 mg. of mercaptan diffused in 230 cubic metres of air is still distinctly perceptible. In this case a litre of air would contain only ·00000004 mg. of the substance, and the amount actually in contact with the olfactory epithelium would be still smaller. It is possible, however, to show the presence of these odorous substances in air by physical means. Tyndall pointed out that air containing a small proportion of odorous substances absorbed radiant heat to a much greater degree than did pure air. Thus in one experiment, air containing patchouli absorbed radiant heat thirty-two times as strongly as the pure air. Most odorous substances possess large molecules and have therefore high vapour densities. On this account the smell tends to hang about objects, the rate of diffusion of the vapour being only small.

Mode of Action of Odours. Since the endings of the olfactory cells are bathed in fluid, it is evident that the odorous substances must be dissolved by this fluid before they can excite the olfactory nerve fibres, and in the case of aquatic animals we know that the projected chemical sense, which we call smell, can be aroused only by substances in solution. It is difficult to show in man that the nerve endings can be excited by solutions. Most of the experiments have been made with solutions which had an injurious effect upon the olfactory epithelium. According to Aronsohn it is possible to excite sensations of smell if the nasal cavity be filled with normal saline fluid containing a very small proportion of the odorous substance.

Many olfactory stimuli owe their peculiar character to the simultaneous stimulation of other kinds of nerve endings. Thus a pungent smell, as that of ammonia, chlorine, &c., besides stimulation of the olfactory nerve, involves stimulation of the nerve endings of tactile sensibility, supplied by the fifth nerve.

OLFACTOMETRY. Elsburg, Levy and Brewer have recently devised a satisfactory method of measuring the acuity of smell in different people. A bottle containing the odorous substance can be filled with air at a slightly raised pressure so that on releasing a valve a known volume of air containing the odour is injected into the nasal cavities. For different odorous substances, different volumes of air must be used to reach the threshold, as the following table shows :—

Benzene	5·26 c.c.
Xylene	10·0 ,,
Camphor	15·0 ,,
Clove oil	17·22 ,,
Cedar wood oil	32·65 ,,

They have found that in women just prior to menstruation the acuity of the sense of smell is increased. A cold in the head depresses it often for several days. Smelling a substance depresses for a time the acuity for that substance.

THE CLASSIFICATION OF SMELLS. No satisfactory classification of smells has yet been made. The following facts tend to show that there are a number of primitive sensations of smell, as of other sensations :

(*a*) Certain individuals, whose olfactory sense is in other respects normal, have no power of distinguishing some odours.

(*b*) The olfactory sense is easily fatigued. If it be fatigued so as to be absolutely insensitive for one kind of smell, it is still normally excitable for other smells.

(*c*) It is possible by mixing odoriferous substances in certain proportions to annul their effect on the olfactory organ. Thus 4 grm. of iodoform in 200 grm. of Peruvian balsam is almost odourless, and the same neutralisation of odours is obtained if the odour of each substance be allowed to act separately on each side by tubes inserted into each nostril.

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BOOK V

BLOOD, CIRCULATION AND RESPIRATION

CHAPTER XXIX

THE BLOOD

In all the higher animals, the blood circulates through a closed system of tubes and is separated from the tissue elements themselves by the walls of the blood vessels. The free interchange of material between blood and tissues is facilitated by the tenuity of the capillary wall. The interstices of the tissues contain a fluid, the 'tissue fluid,' which is formed by the transudation of materials from the blood through the walls of the capillaries, and the interchange between the blood and the tissue cells is effected through this fluid. In a similar manner, excess of water and certain dissolved constituents of the tissue fluid freely pass into special channels, known as the lymphatics, and by these are returned again to the blood. Since the function of the blood is to act as the common nutritive medium of all parts of the body, it has to convey food materials and oxygen to the tissues. From these it receives in exchange their waste products, and carries them away to the excretory organs, such as the lungs and kidneys, by which they are eliminated. It is evident that the composition of the blood must vary from time to time and place to place according to the condition of activity and the function of the organ which it is traversing. The organs of the body are adjusted to respond to very minute changes in the composition of the circulating fluid, and add to or subtract from its constituents according as these are present in deficiency or excess. The changes are therefore kept within infinitesimal limits and we may therefore treat the blood as a fluid of approximately constant composition and qualities.

Blood obtained from a mammal is an opaque fluid varying in tint according to the vessel from which it is derived, being scarlet when taken from an artery, purplish in colour when taken from a vein, the difference being determined by the degree of oxygenation of the blood. On shaking venous blood with air, it takes up oxygen and acquires the scarlet colour characteristic of arterial blood. If examined in a thin layer under the microscope, its opacity is seen to be due to the fact that it is not homogeneous, but consists of a number of *corpuscles* of different kinds, suspended in about their own bulk of a light yellow transparent fluid, the *plasma*. It is seen to contain three main classes of corpuscles. Much the most numerous and conspicuous are the *red corpuscles*. Next most conspicuous are the *white corpuscles*; the third type of formed elements are very small and are called the *blood platelets*. The average numbers of these formed elements per cubic millimetre of normal human blood are as follows:

Red corpuscles	5,400,000
White corpuscles	10,000
Platelets	300,000 (variable).

In the whole body there are about 32 billion red corpuscles and about 50,000 million white corpuscles. The total red cell volume of the body is about 2 l., or 30 c.c./kg. body weight.

Unless special precautions are taken, the examination of blood obtained from a blood vessel is interfered with by the process of clotting, which ensues shortly after the blood has left the vessels. If blood be received

into a beaker it is at first perfectly fluid. After a space of time, varying from three to eight minutes, it begins to be viscous, and if poured out of the beaker leaves an adherent layer on the sides of the vessel. A minute later the whole mass of the blood becomes solid and the beaker can be inverted without spilling its contents. If a section be made of this blood clot, it is found to owe its solidity to a network of fine threads of a protein substance named *fibrin*, which have formed throughout the plasma and enclosed the corpuscles in their meshes (Fig. 307). On leaving the clot for some hours, drops of yellow fluid appear on its surface and run together. The whole clot contracts, and finally there is a reduced clot, floating in a transparent yellowish fluid known as *serum*. If the blood on leaving the vessels be whipped with a bunch of twigs, or stirred with a glass rod, the filaments of fibrin as they are formed are deposited on the stirrer. After three or four minutes the twigs can be withdrawn and the spongy fibrin collected. The blood which is left consists only of the corpuscles *plus* serum, and it will not clot since its fibrin has been removed. It is known as *defibrinated* blood. Since clotting can be produced in blood plasma entirely separated from corpuscles, we must look upon the process of coagulation as determined in the main by changes in the blood plasma. We can regard the blood as a tissue consisting of a fluid matrix, which is extremely unstable and undergoes change when it leaves the vessels, and as having floating in its matrix formed elements or cells of various kinds.

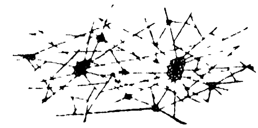

FIG. 307 Network of Fibrin, after washing away the corpuscles from a film of blood that has been allowed to clot; many of the filaments radiate from little clumps of blood platelets. (SCHAFER)

THE QUANTITY AND COMPOSITION OF THE BLOOD IN MAN

The Total Blood Volume. The amount of blood contained in the body can be estimated by Welcker's method, in which the animal is bled and the residual blood is washed out from the vessels with salt solution. The hæmoglobin content of blood *plus* washings is then determined and compared with that of a specimen of the undiluted blood. In this way it was found that the dog contains about 7·7 per cent. of his body weight as circulating blood, and the same has been taken as applicable to man, on the basis of two observations made in 1856 on executed criminals.

Modern methods, applicable also to man, involve the addition to the blood of a known amount of some substance which does not quickly leave the blood stream, and the subsequent estimation of the substance in the blood after thorough mixing has occurred. The substances used are carbon monoxide, or one of several dyes. The former gives the volume of the whole blood, the latter that of the plasma, to which the corpuscular volume, determined by the hæmatocrit, must be added.

The carbon monoxide method* depends on the fact that carbon monoxide gas when inhaled combines with hæmoglobin. It therefore depends on the determination of available hæmoglobin which is accessible to the circulation. It probably does not include that present in the corpuscles which are stored in the spleen, though sometimes there may be some increase from this source.†

* HALDANE and LORRAIN SMITH. *J. Physiol.*, 1900, **25**, 331. A modified form has been described by CHANG and HARROP, *J. Clin. Investig.*, 1928, **5**, 393.

† WATERFIELD. *J Physiol.*, 1931, **72**, 117.

If we allow a man to rebreathe air containing a known volume of carbon monoxide until it is entirely absorbed, and then find that, say, one-fifth of the hæmoglobin in his blood is saturated with carbon monoxide, we know that the whole blood could take up five times the bulk of carbon monoxide which the man has inspired. In this way we determine the total 'carbon monoxide capacity' of all his blood, and since CO-hæmoglobin contains the same volume of carbon monoxide as oxyhæmoglobin does of oxygen, the same figure gives us the total 'oxygen capacity.' If we know the percentage oxygen capacity of the blood, it is easy to calculate the total volume of circulating fluid. The estimation probably gives too high a result, because some of the CO would be fixed by the myoglobin of the muscles. The error is probably not greater than 10 per cent.

The dye method of determining the blood volume was worked out by Keith, Rowntree and Geraghty,* The method consists in injecting a non-toxic, non-diffusable dye substance into the blood stream and estimating its dilution. The difficulty lies in finding a suitable dye, since many dyes easily diffuse out of the circulation, while colloidal ones are liable to be taken up by the cells of the reticulo-endothelial system. In either case the estimated blood volume would be too high. The dye used is 'brilliant vital red,' or Evans Blue (T 1824).† In performing the test a few c.c. of blood are removed from an elbow vein into a syringe containing one-fourth of its volume of isotonic (1·6 per cent.) sodium oxalate to prevent clotting. About 0·2 c.c. per kg. body weight of a 1 per cent. solution of the dye in distilled water is then slowly injected by the same needle. Four minutes later a second specimen of blood is withdrawn into a quarter of its volume of oxalate solution. A part of this sample is drawn into a hæmatocrit tube and centrifuged for twenty minutes at a high speed in order to determine the relative volume of corpuscles and plasma. The rest of both samples of blood are centrifuged in order to obtain the plasma. These samples of plasma are then compared by preparing the following mixtures :—

Standard	1 part of the diluted dye solution ($\frac{1}{267}$ per cent.). 1 part of the plasma before dye injection. 1 part 0·9 per cent. NaCl solution.
Test	2 c.c. of plasma after dye injection. 4 c.c. 0.9 per cent. NaCl solution.

The two solutions are compared in a colorimeter (preferably photoelectric ‡) and the test solution read off as a percentage of the standard. The following formula will give the plasma volume :—

If R be the concentration of test solution as per cent. of the standard, D be the c.c. of 1 per cent. dye injected and C be the ratio c.c. plasma/c.c. plasma and oxalate, then,

$$\frac{26700 \text{ D.C.}}{\text{R}} = \text{c.c. plasma.}$$

The blood volume is calculated from the hæmatocrit reading.

$$\text{Total blood volume} = \frac{100 \times \text{c.c. plasma}}{\text{percentage plasma in blood}}.$$

Estimates of blood volume vary from $\frac{1}{10}$th to $\frac{1}{20}$th of the body weight. The CO method gives 5 to 7 per cent. of body weight, the dye method 7 to

* KEITH, ROWNTREE and GERAGHTY. *Arch. Int. Med.*, 1915, **16**, 547.

† DAWSON, EVANS and WHIPPLE. *Amer. J. Physiol.*, 1920, **51**, 232 ; GIBSON and EVANS *J. Clin. Investig.*, 1937, **16**, 301.

‡ KENNEDY and MILLIKAN. *J. Physiol.*, 1938, **93**, 276.

10 per cent. of body weight. As a round figure we may take 6 l. as the blood volume of a 70 kg. adult. The volume probably is more nearly proportional to body surface than to body weight, a rough figure being 2500 c.c. blood per sq. m. surface (CO method) or 3200 c.c./sq. m. (dye method). The volume per sq. m. is equal in the two sexes, increasing from infancy to adolescence, and also increasing in pregnancy.

The total blood volume certainly varies slightly with alterations in the condition of the animal, *e.g.*, in thirst, and may be found different on two succeeding days. It is influenced by posture, there being a loss of about 15 per cent. of the total plasma in the erect position as compared with the horizontal.* It is also affected by the height of the blood pressure as well as by the oxygen tension in the air breathed, and therefore alters with the altitude. Some of these variations we shall have to consider more fully in a later section. Any lowering of blood pressure causes an absorption of fluid from the tissues into the blood, so that the latter becomes more dilute. The blood during the last stages of bleeding may contain little more than 50 per cent. of the corpuscles which were present in the first samples of blood, pointing to a corresponding dilution of the blood during these few minutes by means of tissue fluid. By this means, *i.e.* the absorption of fluid from tissues, the volume of circulating blood after a limited hæmorrhage is rapidly brought up to normal, so that there is a circulation of fluid impoverished in corpuscles.

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DETERMINATION OF THE RELATIVE VOLUMES OF CORPUSCLES AND PLASMA. The relative volumes of corpuscles and plasma in any sample of blood may readily be determined by means of the *hæmatocrit* (Fig. 308).† This is a graduated capillary tube of uniform bore in which the specimen of blood, treated with a suitable anti-coagulant, is centrifuged at a high speed until the sedimented corpuscular layer shows no further shrinkage, and appears translucent. The depths of the layers of corpuscles and plasma are then read off, and are expressed as percentages of the whole length of the column of blood taken. It is usually found that in normal human blood the corpuscles occupy about 40 to 50 per cent. of the volume of the blood.

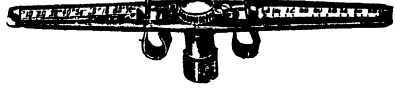

FIG. 308.—Hæmatocrit. (From HARRIS' "Experimental Physiology.")

The number of red cells in the circulating blood is rapidly variable in two ways, viz. by alterations in the quantity of plasma in circulation, as mentioned above, and by variation in the total number of red cells in circulation. The latter may alter abruptly in consequence of the storage in, or liberation of, red cells from the spleen. When the spleen contracts, a considerable number of stored-up red cells are discharged into general circulation.‡

* WATERFIELD. *J. Physiol.*, 1931, **72**, 110.

† HEDIN. *Skand. Arch. Physiol.*, 1891, **2**, 134, 360; HIROTA. *J. Biophys.*, 1925, I., 233.

‡ BARCROFT *et al.* *J. Physiol.*, 1925, **60**, 443.

Cell Count and Hæmoglobin Content. In order to enumerate the red corpuscles, the blood is diluted with a known amount of an isotonic fluid and the number is counted in a measured volume of the mixture by means of a hæmocytometer. The average number (*v.* Fig. 309) of red corpuscles is, about 5,400,000 per cubic millimetre in healthy adult men and rather fewer and more variable, in adult women. The enumeration of corpuscles is subject to considerable errors, rarely less than 5 per cent. Moreover, different conditions of the circulation may cause variations in the relative distribution of plasma and corpuscles respectively in different parts of the circulation, so that the blood-count of a specimen from the capillaries of the finger or lobe of the ear may differ considerably from a similar count of the corpuscles in blood obtained directly from a vein or artery.

More important, therefore, is the determination of the hæmoglobin. For this purpose, a measured quantity of the blood, say, 20 c.mm., is mixed with a given volume of water. The red fluid thus obtained is compared in a colorimeter, or a specially designed "hæmoglobinometer," with a standard. Usually the hæmoglobin is converted into a more deeply coloured and more

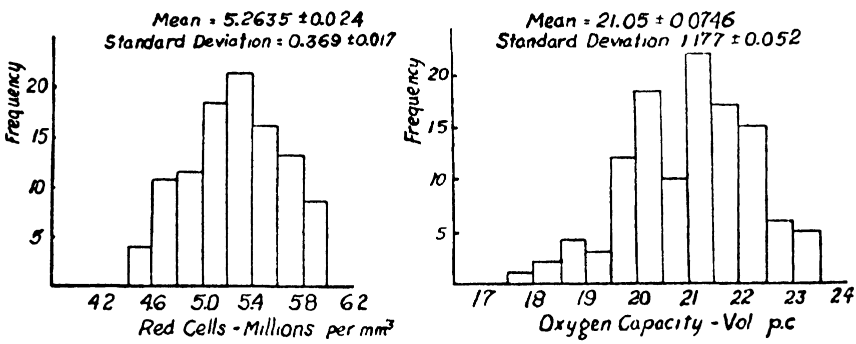

FIG. 309. Showing statistical grouping of red cell count and oxygen capacity in American male students, age 18–30. (FOSTER and RAYMOND JOHNSON.)

stable derivative. In Sahlis' method the blood is diluted with 0·1 N.HCl, whereby the hæmoglobin is converted into acid hæmatin,* Haldane's method † depends upon the conversion of the hæmoglobin into CO-hæmoglobin, and its comparison with a standard specimen of CO-hæmoglobin, which is stable in solution. Haldane's standard normal hæmoglobin in human blood is one with an oxygen capacity of 18·5 volumes per cent. This hæmoglobin content is called 100 per cent. on the Haldane hæmoglobinometer. This standard is largely used in clinical work in Great Britain. Normal adult males usually have about 114 per cent., and females about 100 on the Haldane scale. The practice of stating the hæmoglobin in terms of g. Hb per 100 c.c. blood, or of oxygen capacity, is increasing.

Another method is to determine the iron content of the blood,‡ since hæmoglobin contains 0·0335 per cent. Fe.

An important clinical determination is that of the *Colour Index* of the blood. For this a red cell count and a hæmoglobin determination are needed. Then, calling 5 million red cells 100 per cent., the colour index $= \frac{\text{per cent. Hb}}{\text{per cent. cells}}$. This index, which normally is 1·0, shows the relative hæmoglobin content of the average corpuscle.

* COHEN and SMITH. *J. Biol. Chem.*, 1919, **39**, 489.
† HALDANE. *J. Physiol.*, 1900, **26**, 497.
‡ WANG. *J. Biol. Chem.*, 1928, **77**, 409.

The Oxygen Capacity of the Blood. Instead of determining the hæmoglobin, we may measure directly the oxygen capacity of the blood, since the oxygen-binding power of this fluid is entirely dependent on the amount of hæmoglobin it contains. For this purpose we may make use of the fact discovered by Haldane, that the combined oxygen in oxyhæmoglobin is liberated rapidly and completely on addition of a solution of potassium ferricyanide to alkaline laked blood, and may thus be easily measured.

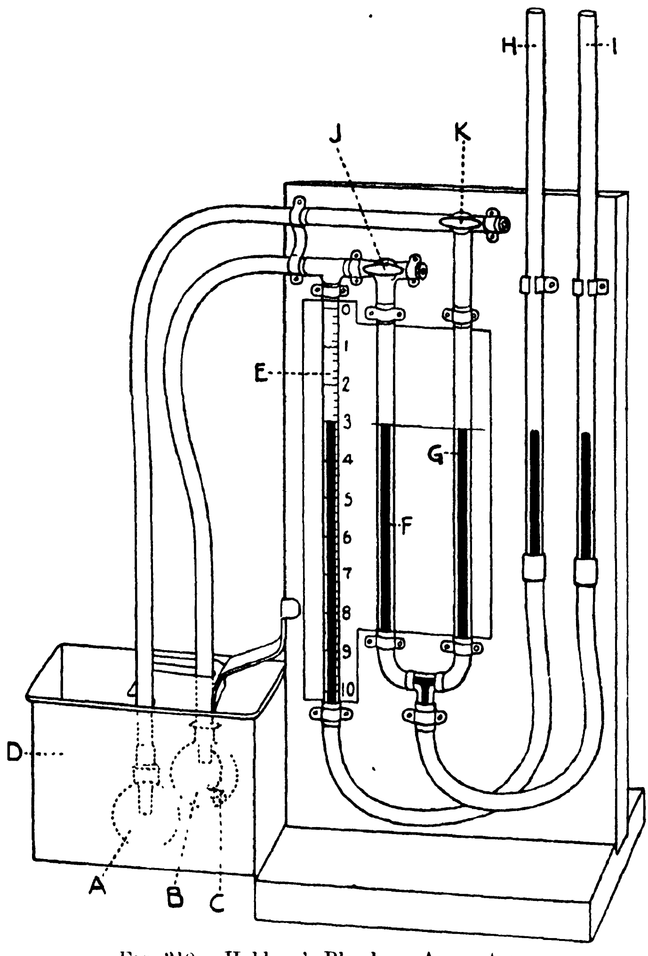

FIG. 310. Haldane's Blood-gas Apparatus.

For this purpose the blood-gas apparatus of Haldane is convenient (Fig. 310).

Into the flask B is placed 2 c.c. of 1 per cent. sodium carbonate solution and a few milligrams of saponin. The blood, (2 c.c.), rendered incoagulable with oxalate, is run into it, and well mixed, laked, and oxygenated by rotating in the flask for a few minutes. Then 0·25 c.c. of saturated ferricyanide solution is put into the small tube C, and the cork inserted, the control flask A being similarly corked, and both flasks placed under water in the bath D, the three-way taps J and K being open to the air with the T bore thus : T. After allowing a few minutes to attain temperature equilibrium, the levelling tubes H and I (filled with water containing a little saponin or bile-salts) are adjusted so as to bring the water in F and G to the marks, and the taps J and K are then turned with the bore thus : ⊣. The burette E is then read, the flask B is tilted so that the ferricyanide

runs into the blood solution, and the flask is shaken in the bath until no more gas is evolved. After allowing a few minutes to equalise any differences of temperature, the water level is again adjusted in F and G, and the volume of gas evolved read off on the burette E, and reduced to dry volume at N.T.P.

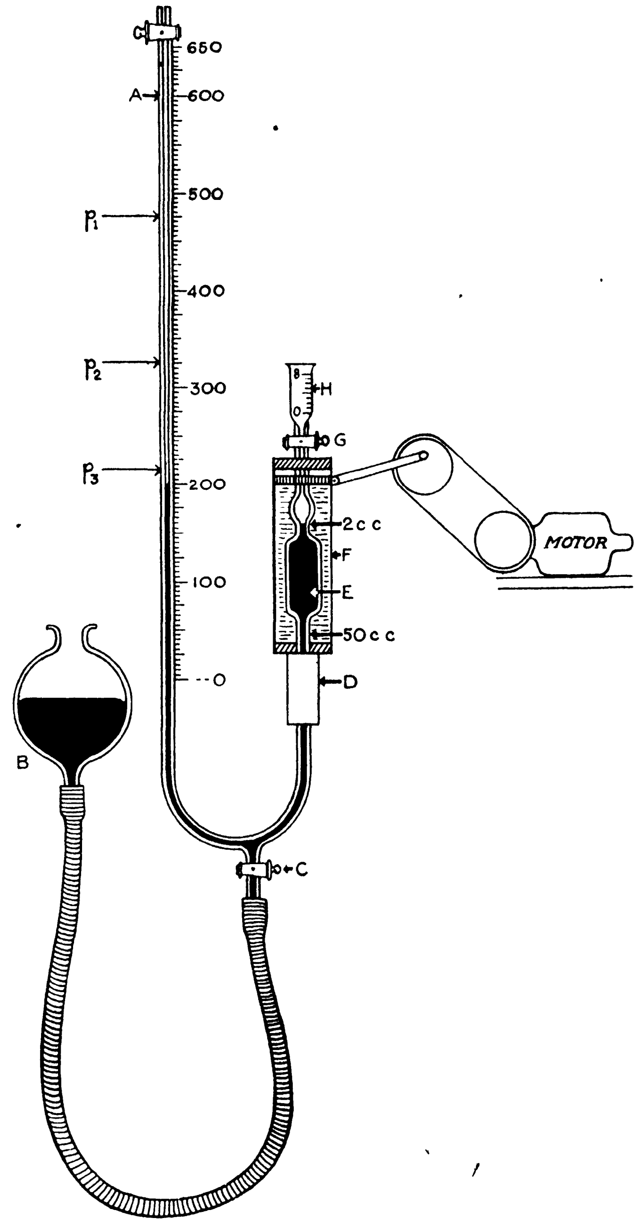

FIG. 311. Diagram to illustrate the principle of the Van Slyke manometric apparatus.

For more accurate work and also for determining the CO_2 content of the blood the Van Slyke apparatus * or its modern manometric form † is valuable.

* VAN SLYKE and STADIE. *J. Biol. Chem.*, 1921, **49**, 1.
† VAN SLYKE. *J. Biol. Chem.*, 1927, **73**, 121.

The manometric Van Slyke apparatus depends on the principle of measuring the *pressure* of gases evolved or absorbed in a chamber of a *given volume*. From these pressures, or pressure differences, the volumes, or volume differences at N.T.P., can be calculated in accordance with Boyle's law. The *principle* of the apparatus is illustrated by Fig. 311, from which the actual apparatus differs only in some unessential details. The tube A, graduated in mm. is a barometer filled with dry mercury, and connected by a U-shaped bend with the extraction chamber E, junction being effected by a thick-walled rubber tube D. The extraction chamber is graduated at three marks, from the tap, viz. 0·5 c.c. to 2 c.c., and 50 c.c., and can be filled or emptied of mercury by raising or lowering the bulb B. If it is filled with mercury, and the tap G closed, lowering of the bulb to a low enough level will produce a vacuum in the chamber. By manipulating the level of the mercury bulb and the tap C, the vacuum can be made of any volume desired, *e.g.*, the mercury can be brought exactly to the 2 c.c. mark. If the mercury in the chamber was dry, the levels in the chamber and in the barometer will now be equal; if it was wetted with water this will exert a vapour pressure dependent on the temperature, and the barometer will be higher by that amount, *e.g.*, by 17 5 mm. at 20° C. Suppose that we were now to introduce into the chamber (at 0° C.) 0 5 c.c. of a gas consisting of, say, 10 per cent. CO_2, 20 per cent. O_2 and 70 per cent. N_2, and by admitting mercury adjust the volume of this again to 2 c.c. Then the mercury would rise by $\frac{760 \times 0{\cdot}5}{2{\cdot}0}$ mm. = 190 mm. in the barometer. Suppose that now we admit by the tap G a small amount of a NaOH solution to absorb the CO_2, and again bring the volume to 2 c.c. We should find the pressure had fallen to 171 mm. above the "wet level." Similarly, after absorbing the oxygen by hydrosulphite solution the pressure would fall to 133 mm. above the vacuous wet level.

Evidently the composition of the gas could have been deduced from these readings, and also the amounts at N.T.P. Thus the pressure of oxygen would be 171 − 133 = 38 mm., and its volume would be $\frac{2 \times 38}{760} = 0{\cdot}1$ c.c., which we know to be correct.

In using the apparatus for the estimation of the gases in blood an acidified ferricyanide solution is first introduced into the chamber, which is then evacuated and shaken by the motor attachment, to extract all dissolved air. A measured volume of blood (1 c.c.) is then introduced through tap G, the chamber again evacuated and shaken, and thus all gas expelled from the blood, the CO_2 by the acid, the oxygen by the ferricyanide, and the nitrogen by the evacuation. The volume of gas is then brought to 2 c.c., and the pressure p_1 is read. Then 1 c.c. of N·NaOH is admitted to absorb CO_2, the volume again brought to 2 c.c., and pressure p_2 is read. From $p_1 - p_2$ with certain corrections, the volume of CO_2 is found. Next 1 c.c. of an alkaline hydrosulphite is introduced, volume brought to 2 c.c. and p_3 read. Oxygen is calculated from $p_2 - p_3$. For very small volumes of blood the gases may be read at 0·5 c.c., so that pressures four times as great are given throughout. In practice various precautions have to be taken, but the calculations are greatly simplified by the use of appropriate tables.*

AVERAGE RED CELL DATA FOR HUMAN BLOOD. The data accepted for average human red cell count and hæmoglobin content at the beginning of the present century appear now to be too low.

Price-Jones † found as follows for 100 healthy men and women in London :

	Men (average age 27).	Women (average age 24)
Red cells, p.c.mm.	5·428 million	5·012 million
Hæmoglobin, p.c. (Haldane).	105·42 ,,	98·26 ,,
Colour Index $\left[= \frac{Hb\% \times 5}{\text{Millions of cells per c.m.m.} \times 100}\right]$.	0·97 ,,	0·98 ,,

The following data, averaged from 115 healthy American male students, aged 18 to 30, are given by Foster and Raymond Johnson (1931)‡ (*v.* Fig. 309).

(1) Red cells = 5·26 million, p.c. mm.

(2) Hæmoglobin = 15·63 grams per cent.

* PETERS and VAN SLYKE. Quantitative Clinical Chemistry., 1932. (Baillière, Tindall and Cox).

† PRICE-JONES. *J. Path. and Bact.*, 1931, **34**, 779.

‡ FOSTER and RAYMOND JOHNSON. *Proc. Soc. Exp. Biol. Med.*, 1931, **28**, 929.

(3) Oxygen capacity = 21 05 vols per cent. (= 114 per cent. on Haldane scale and Colour Index = 1·08).

(4) Hæmatocrit volume : 46·7 per cent. (Heparinized.)

(5) Average volume of Corpuscle = 88 8 μ^3 per corpuscle $\left(= \frac{(4)}{100 \times (1)}\right)$.

(6) Average corpuscular Hb = 30 03 $\times$ 10^{-12} grams $\left(= \frac{(2)}{(1) \times 10^5}\right)$.

(7) Relative Corpuscular Hb = 34 77 per cent. $\left(= \frac{(6)}{(5)}\right)$.

THE SPECIFIC GRAVITY OF THE BLOOD. The specific gravity of the blood may be determined by collecting blood in a capillary tube and discharging drops of it into a liquid of known specific gravity, with which it will not mix. Thus, we can use a mixture of chloroform and benzene and then add chloroform or benzene, as the case may be, until the drop neither rises nor falls. The specific gravity of the mixture is then taken. The specific gravity varies between 1055 and 1066, being lowest in women. It is increased by loss of water, as after profuse perspiration, or by passive congestion of the part from which the sample is taken. It is also increased as a result of any operation upon a serous cavity in consequence of exudation of plasma in the inflamed or irritated part. It is diminished as the result of bleeding. The specific gravity of serum is 1028 to 1032, of corpuscles about 1090. It is interesting to note that the specific gravity of the blood is highest in the fœtus at full term, when it amounts to 1066, contrasting with that of the mother at the same time, the specific gravity of whose blood is only 1050. The specific gravity rapidly falls after birth.

VISCOSITY OF THE BLOOD. Blood is more viscous than water, but since it is not a homogeneous liquid its apparent viscosity varies according to the method used for the measurement. Thus when viscosity is determined by the rate of flow along a capillary tube, the apparent viscosity is increased by increase in the relative number of corpuscles, by diminution in the bore of the capillary and by reduction in the driving pressure A figure commonly given is five times that of water.

THE SUSPENSION STABILITY OF THE BLOOD. From the size of the red cells and the difference in specific gravity between them and the plasma, it can be calculated that when blood is allowed to stand the corpuscles should sink at a speed of about 0·2 mm. per hour; yet the experience of centuries has shown that the speed with which sedimentation occurs is far more rapid and shows enormous variations, being greatly increased in some disorders.

In the days when blood-letting was almost universal in the treatment of disease, it was a frequent observation that on allowing the blood to stand there was formed at the surface of the clot a pale layer, known as the *buffy coat.* This was due to the fact that the corpuscles have settled so rapidly that the clot is formed in part in the supernatant plasma, and is, therefore, pale in colour.

Fåhræus studied the sedimentation of citrated blood by allowing samples to stand undisturbed in long narrow tubes, and measuring the length of clear plasma after one hour. The following average results were obtained :—

New-born children	0·5 mm. per hour.
Normal men	3·3 ,, ,,
Non-pregnant women	7·4 ,, ,,
Pregnant women at term	44·9 ,, ,,

The increased rate of sedimentation is due to the phenomenon known as *agglutination,* which consists in the corpuscles aggregating together into clumps. In blood with a sedimentation value of 1 mm., each clump consists of about eleven corpuscles, while one with a value of 75 consists of clumps each composed of about 58,000 corpuscles. If the corpuscles and plasmata be separated off from two bloods with very different suspension stabilities, *e.g.* normal male and gravid female bloods, and then exchanged, it is found that the speed of settling is determined by some constituent of the plasma and not of the corpuscles. The logarithm of the maximal sedimentation velocity is proportional to the concentration, in the plasma, of the specific substances causing agglutination. This constituent is usually associated with the globulin fraction.

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PRICE-JONES *et al.* (1935). Hæmatological Standards of Healthy Persons. *J. Path. & Bact.*, **40**, 503.

Blood Groups. The phenomenon of agglutination is often shown in an exaggerated form when the blood of one species is added to the blood, or to the plasma or serum, of another. If, for instance, a drop of human blood be mixed, on a slide or white plate, with some horse serum, the agglutination is at once evident to the unaided eye. It is found that human beings fall into four groups, when agglutination tests are performed.

Agglutination is due to the interaction between substances in the corpuscles, called *agglutinogens*, and substances called *agglutinins* in the plasma. Two main agglutinogens, called A and B, may exist in human red cells, and these can react with two agglutinins, called α and β, found in human plasma or serum. The cells of an individual may contain one or both agglutinogens, or none at all, and similarly for the agglutinins of the serum. Subjects thus fall into four groups, according as the cells are A, B, AB (*i.e.* with both) or O (*i.e.* with neither). The blood with A cells contains the β plasma, that with B contains α, that with AB contains neither agglutinin, and that with the O group of cells contains both α and β agglutinins in the plasma. Hence when the sera of one group are mixed with cells of other groups the results are as shown in the table (+ indicates agglutination).*

Corpuscle Group	Serum Group	Effect of adding Serum of Group			
		O	α	β	$\alpha\beta$
A	β	—	+	—	+
B	α	—	—	+	+
AB	O	—	+	+	+
O	$\alpha\beta$	—	—	—	—

The corpuscles of Group O are not agglutinated by the plasma of any group, and Group O are accordingly called 'Universal Donors,' since their blood is safe for transfusion into any person. In Group AB the plasma is O, and does not agglutinate any corpuscles, so that people belonging to this group could receive blood from any healthy person. The other groups are as shown. Thus Group B could act as a donor to Groups AB or B only.

It is usual to carry out grouping tests by the use of tested sera belonging to Groups A and B. Drops of the citrated blood of the person to be tested are mixed on a white plate with a drop of each of these, and, as in the table :

Group AB is agglutinated by A and B.
Group A ,, ,, B
Group B ,, ,, A
Group O ,, ,, neither.

Among English people, about 2 per cent. belong to Group AB, 40 to Group A, 15 to Group B, and 43 to Group O. With other races the distri-

* The above is the League of Nations grouping Other groupings often used are that due to Moss, whose Groups I, II, III and IV correspond respectively to AB, A, B and O of the above and that of Jansky, largely used in U S A , where, in the same order, the groups are numbered IV, II, III, and I.

bution is different.* The agglutinogens are passed on by inheritance as Mendelian dominants, the agglutinins as recessives—a fact of medico-legal importance in cases of disputed paternity.†

The classification of blood groups is made still more precise, especially in examination of medico-legal cases, by the discovery that, quite independently of the AB system mentioned above, another classification can be made, based on different agglutinogens called M and N. These are identified by special immune rabbit sera, and provide three independent types, M, N and MN. As these occur independently of the AB groups, the combination yields twelve possible blood types, and in fact, yet other subsidiary groups are known.

BLOOD TRANSFUSION. The facts of blood grouping acquire great practical importance, because agglutination is the precursor of hæmolysis, and because the plasma of one human being frequently causes agglutination of the corpuscles of another. In carrying out the urgent operation of blood transfusion, it is therefore a matter of great importance to ensure that the corpuscles of the donor will not be agglutinated by the plasma of the recipient. To make quite sure of this, it is necessary, if circumstances allow, to test out the blood of the donor directly against the serum of the recipient, in addition to the grouping test, because some corpuscles may in spite of identity of main groups, belong to subsidiary groups and prove to be incompatible with a given serum. Further essential precautions include the avoidance of clotting, and of infection, and the introduction of blood at the proper temperature, and at the proper rate, into the veins of the recipient. Coagulation is usually prevented by the addition of sodium citrate, 3·2 per cent. solution, to five times its volume of blood. The citrated blood can be preserved at 5° C. for periods up to ten days, or longer, if an equal volume of 5·4 per cent. glucose should be added to the mixture. Collections of stored blood (blood banks), duly grouped, are often kept at large hospitals. When hæmolysis begins to set in rapidly the stored blood must be rejected.

Transfusions of incompatible blood may lead to severe symptoms and may be fatal. The incompatible corpuscles are hæmolysed in the body, hæmoglobin is excreted in the urine, or the kidneys may even cease to function, and a state of shock and collapse ensue.

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The Reaction of the Blood. The blood is slightly alkaline to litmus, and can be titrated with an acid. Such a procedure, however, gives a measure, not of the degree of alkalinity, but of the alkaline reserve—*i.e.* of the total amount of base in combination with weak acids which can be replaced by a stronger acid. This *alkaline reserve* consists almost exclusively of sodium bicarbonate.

In Van Slyke's method the alkaline reserve of the plasma is determined by finding out how much CO_2 is evolved from 1 c.c. of the oxalated plasma, when this is treated with 0·5 c.c. of 5 per cent. sulphuric acid. Since the amount of carbon dioxide taken up depends on the partial pressure of the CO_2 in the atmosphere to which the plasma is exposed, the plasma is first shaken up with alveolar air provided by the experimenter himself, which always contains about 5·5 per cent. of CO_2.

Normal human blood plasma treated in this way yields between 60 and 70 c.c. of CO_2 per 100 c.c., which corresponds to about 0·2 per cent. of sodium bicarbonate.

The *hydrogen ion concentration* of the blood can be determined by the electrometric method, or by the use of indicators, as described on p. 70.

By these methods the hydrogen ion concentration of the blood, *i.e.* of the plasma, at 38° is found to be $0{\cdot}4 \times 10^{-7}$, or pH 7·4. It might be thought

* MILLOT. *Antiquity*, 1935, 9, 399.
† HARLEY and ROCHE LYNCH. *B.M.J.* 1937 (i), 163; THOMAS. *Ibid.* (ii), 315.

that with such a feeble alkalinity the merest trace of acid added to the blood would suffice to make it acid. It is found, however, that a relatively large proportion of an acid must be added to the blood in order to produce a considerable change in its reaction. This is due to the fact that the sodium bicarbonate acts as a ' buffer '—*i.e.* a substance which can take up acid or alkali with a minimal change of reaction. As in other buffer systems (*v.* p. 68) the hydrogen ion concentration depends on the ratio of the weak acid to its salt, *i.e.* to $\frac{H_2CO_3}{NaHCO_3}$. This in blood is usually about $\frac{1}{20}$ when molar concentrations are taken. This property of the blood of retaining a nearly constant reaction, even though fixed acids are added to it, is of immense importance, since all cellular functions are acutely sensitive to changes in reaction, and as we shall see later, the activity of the respiratory centre is largely dependent on the hydrogen ion concentration of the blood with which it is bathed.

The alkaline reserve of the blood is significant, since pronounced diminution indicates in all probability the production of fixed acids in the tissues, and a progressive reduction will precede the point at which the ' buffer ' action of the sodium bicarbonate is lost, and the blood then responds to any addition of acid by a considerable change in reaction. It is only when the alkaline reserve has been greatly reduced that a true condition of *acidæmia*, or increased H-ion concentration, can come into being.

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THE OSMOTIC PRESSURE OF THE BLOOD. Since the blood serves as a circulating medium, by means of which the composition of the tissue juices is maintained constant, its osmotic pressure must be of considerable importance. The osmotic pressure of the blood depends on its molecular concentration and can be determined by any of the methods mentioned earlier (p. 61), *e.g.* by the determination of the freezing-point. The depression of freezing-point, Δ, of mammalian blood in equilibrium with air is about 0·53°. This is equal to that of a 0 9 per cent. sodium chloride solution, which is therefore often taken as isotonic with the blood.

By the use of Hill's thermopile method for vapour pressure, Margaria * found the average osmotic pressure of the blood of adult men to be equal to that of 0·945 per cent. NaCl (Δ = 0·553° C.) when the blood was in equilibrium with 5 per cent. CO_2. The blood of women had a lower osmotic pressure = 0·927 per cent. NaCl (Δ = 0 543° C.). Under normal conditions the osmotic pressure is remarkably constant; it is reduced slightly by large ingestions of water and increased slightly during severe muscular exercise.

Actually the osmotic pressure of the contents of the red corpuscles is a little lower than that of the plasma, but the difference is not great enough for hæmolysis appreciably to alter the freezing-point or osmotic pressure of the blood.

For frog's blood Hill and Kupalov found an osmotic pressure = 0·725 per cent. NaCl.

THE ELECTRICAL CONDUCTIVITY OF THE BLOOD. In a solution it is only the dissociated ions which have the power of carrying electric charges. The conductivity of blood serum is therefore determined almost entirely by its content in salts. Since this is approximately constant, the conductivity of serum varies within very narrow limits. The conductivity of blood varies, however, within wide limits. The corpuscles present a resistance to the passage of the charged ions and therefore of the electric current through them, so that the larger the number of corpuscles contained in a given specimen of blood the lower will be the conductivity of the latter. Stewart has made use of this fact as a

* MARGARIA. *J. Physiol.*, 1930, **70**, 417.

basis for a method of determining the relative volume of corpuscles and plasma. Owing to the fact that the red cells carry a negative charge, they move towards the anode when an electric current is passed through the blood.

THE GENERAL COMPOSITION OF THE BLOOD

Human blood contains from rather over one-third to one-half of its weight of corpuscles. It contains from 20 to 25 per cent. of solids.

BLOOD PLASMA is resolved by clotting into serum and fibrin. The fibrin forms only 0·2 to 0·4 per cent. of the total weight of blood. The *serum* contains, in 100 parts, 8 to 9 parts of solids, of which 7 to 8 parts consist of proteins, while the salts make up to about 1 part.

The chief salt present in the serum is sodium chloride, and next to this comes sodium bicarbonate, and besides these two we find traces of potassium, sodium and calcium, and phosphates. Ammonia is absent from fresh blood, but after shedding appears in amount up to 0·1 mg./100 c.c. This is formed by deamination of adenylic acid derivatives.*

The anions and cations of the plasma are often expressed in terms of milliequivalents per litre. The total base of the serum in man is kept remarkably constant at about 153 milliequivalents per litre. The distribution of ions is roughly as follows:—

Total cations. m eq /l		Anions. m eq /l
153	. . . . . .	$Cl' = 102$
—	. . . . . .	$HCO_3' = 27$
—	. . . . . .	$HPO_4'' = 2$
—	. . . . . .	Protein$' = 15$
153		146

Traces of fats, cholesterol (about $\frac{1}{3}$ free and $\frac{2}{3}$ as esters), lecithin, glucose, urea, amino-acids and other nitrogenous extractives are constantly found in the serum. Tiny particles called *hæmokonia* are always visible in plasma under dark-ground illumination. They consist of fat. The fats are much increased after a meal rich in them and may give the serum a milky appearance. The yellow colour of the plasma is due to the presence of small amounts of bilirubin, carotene ($C_{40}H_{56}$) and xanthophyll ($C_{40}H_{56}O_1$).

THE RED CORPUSCLES contain from 30 to 40 per cent. total solids. Of the solid constituents, hæmoglobin forms nine-tenths; the other tenth corresponds to the stroma, consisting of stroma protein (nucleo-protein), lecithin, cholesterol and salts. There is a striking contrast between the salts of the corpuscles and those in the plasma, the former consisting chiefly of potassium phosphate, the latter of sodium chloride, which in some animals is entirely wanting in the corpuscles. When shed (citrated) blood is stored, as for blood transfusion, at 2° to 4° C. the K of the plasma rapidly rises to 8 to 10 times its initial value.

The total base concentration in the red cells is greater than that in the serum. The anions are correspondingly less, in accordance with the Donnan equilibrium (p. 89), which tells us that:—

$$\frac{\text{anions (cells)}}{\text{anions (plasma)}} = \frac{\text{cations (plasma)}}{\text{cations (cells)}} = \frac{H^{\bullet}\text{ (plasma)}}{H^{\bullet}\text{ (cells)}} = \frac{Cl'\text{ (cells)}}{Cl'\text{ (plasma)}}, \text{ etc.,}$$

the Donnan ratio $\frac{\text{anions (c)}}{\text{anions (p)}}$ is usually about 0·75.

* CONWAY and COOKE. *Bioch. J.*, 1939, **33**, 457.

The following table shows the average percentage composition of human blood :—

NORMAL HUMAN BLOOD

	Per cent in Whole Blood.	Per cent in Plasma.	Per cent. in Corpuscles.
Total solids	22·0	9·3	34·6
Inorganic solids	0·75	0·8	0·7
Serum albumin	2·8	5·6	—
Serum globulin	0·95	1·9	—
Fibrinogen	0·25	0 4	—
Hæmoglobin	14·5	0·0	29·0
Non-protein nitrogen	·032	·025	·044
Urea nitrogen	0·012	·012	·011
(= urea	0·026)	—	—
Amino nitrogen	0·006	0·005	0·008
Creatinine	0·002	—	—
Creatine	0·005	—	—
Uric acid	0·002	—	—
Apparent glucose (*i.e.*, total reducing power)	0·10	0 09	0·10
True glucose	0·07	0 08	0·06
Lactic acid	0 020	0 025	0 014
Total fatty acids	0·36	0 38	0 35
Total 'lipoids'	—	0 67	—
Fat	0·10	0 14	0 07
'Lecithin'	0 3	0 2	0·4
Cholesterol	0 21	0·22	0 19
Total acetone bodies (as acetone)	0 001	—	—
Sodium	0 20	0·335	0·065
Potassium	0 20	0 01	0·38
Calcium	0 006	0 01	0·001
Magnesium	0 003	0 0025	0·0035
Iron	0·05	0 00	0·10
Chlorine (chloride)	0·29	0·36	0·20
Phosphorus, total	0·045	0 010	0 079
Phosphorus, total acid soluble	0 030	0 0035	0 060
,, inorganic	0 005	0 003	0·006
,, 'lipoid'	0·013	0 007	0·018
Sulphate sulphur	0·0005	—	—
Bicarbonate (as HCO_3)	—	0·15	—
,, (as $NaHCO_3$)	—	0·21	—

The Proteins of the Plasma. The plasma contains a number of different coagulable proteins. We may divide them into those which are the precursors of fibrin and those which remain in the serum.

FIBRINOGEN is a globulin converted on coagulation into fibrin. It is formed in the liver. This is proved by the fact that after excision of the liver the fibrinogen content of the blood slowly declines.* If the circulating blood is defibrinated (by removing it, whipping and re-injecting) the fibrinogen content is restored in a few hours and soon exceeds the normal level when intact animals are used; no restoration occurs if the liver has been previously removed, but rather a continued loss.†

Fibrin. Fibrin is easily obtained by whipping blood as it flows from the vessels with a bundle of wires or twigs, and then washing the stringy threads so obtained. As

* NOLF. *Arch. internat. de physiol.*, 1905, **3**, 1.

† MCMASTER and DRURY, *Proc. Soc. Exp. Biol. Med.*, 1928, **26**, 490; JONES and SMITH, *Amer. J. Physiol.*, 1930, **94**, 144.

prepared in this way it always contains fragments of leucocytes, blood platelets and stromata, which have become entangled in its meshes. In order to prepare fibrin in a pure state, it is necessary to get it by the action of thrombin on a pure solution of fibrinogen. Fibrin is insoluble in water and in dilute salt solutions. It slowly dissolves in 5 per cent. sodium chloride, but is converted in this process into soluble globulins. It is probable that its solution is effected by the agency of minute traces of proteolytic enzymes adherent to the fibrin as it is precipitated. In dilute acid, such as 0·2 per cent. hydrochloric acid, fibrin swells into a clear jelly which very slowly undergoes solution with the formation of acid albumin and proteoses.

THE PROTEINS OF THE SERUM. The serum proteins are generally grouped in two classes: the serum albumins and the serum globulins. All the proteins are precipitated by saturation with ammonium sulphate, but only the globulin by half-saturation. The proportion of globulin to albumin is known as the 'protein quotient.' It can be most readily determined from observations of refractive index and viscosity. It varies in different animals, but in the same individual it is almost constant in the blood, serum, lymph and serous transudation, though the total amounts of protein in these may be very different. Globulins are probably the more important because from them are produced the various anti-bodies, such as precipitins, antitoxins, lysins, agglutinins, etc., which play a large part in the phenomena of immunity (*v.* Chapter XXXVIII). The serum proteins are formed in the liver, and serve not only to maintain the colloid osmotic-pressure of the plasma, but also act as a reserve of protein which is available to the body in time of need.

Serum albumin is the name given to a mixture of proteins * which remains in the serum after half-saturation with ammonium sulphate. It can be precipitated from this by complete saturation with ammonium sulphate. The fraction also contains a glyco-protein called globoglycoid.*

The *serum globulins* are mixtures of globulins precipitated by half-saturation with ammonium sulphate. The precipitate has been divided into three fractions, namely, *euglobulin I*, insoluble in water at *p*H 7, *euglobulin II*, insoluble in water at *p*H 6 (precipitated by CO_2), and *pseudo-globulin*, soluble in water. Pseudo-globulin is, however, not soluble in complete absence of salts, and is therefore thrown down by electro-dialysis, which removes the last traces of salt more efficiently than does ordinary dialysis.

Hardy † showed that with acids or alkalies the globulin forms 'salts' which ionise in solution so that in an electric field the entire mass of protein moves. With neutral salts, globulins form molecular compounds which are stable only in the presence of a comparatively large excess of salt.

THE CONDITION OF THE PROTEINS IN THE BLOOD SERUM. Although it is easy by such simple means as the addition or removal of neutral salts roughly to separate one or more different forms of protein from serum, we have evidence that these proteins in the serum are present in a somewhat different form. When examined by the ultra-centrifuge there is found to be less of the large molecules resembling globulin than would be expected from the analysis by salting-out, and correspondingly more of the smaller molecules of albumin dimensions.‡ On dilution this distribution is altered until finally it agrees with the proportions found on analytical separation. Ultra-centrifugation studies also show the presence of molecules of intermediate size, corresponding neither to albumin nor to globulin.

Serum can be readily filtered through a porous cell, and this method is adopted for obtaining it free from contamination by micro-organisms; yet globulin when isolated in solutions will not pass through a porous cell.

* HEWITT. *Bioch. J.*, 1938, **32**, 26.
† HARDY. *J. Physiol.*, 1905, **33**, 251.
‡ MCFARLANE. *Bioch. J.*, 1935, **29**, 407, 660, 1175, 1209.

In explanation of these and other facts, it is now considered that the serum proteins consist of numerous closely related substances, separable roughly into the various groups, *e.g.*, albumin, the globulins, etc., but each member of which is in fact an association of mutually interacting systems of colloids.* By simple means, such as dialysis, dilution or addition of salt, electrophoresis at given *p*H, etc., these complexes can be broken up, with the separation of the various proteins which we have designated as serum albumin and serum globulin, &c.

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MADDEN and WHIPPLE (1940). Plasma Proteins. *Physiol. Rev.*, **20**, 194.

THE RED BLOOD CORPUSCLES

The red blood corpuscles, or *erythrocytes*, in man are non-nucleated bi concave discs, which when in the body are about 8·8 μ in diameter and about

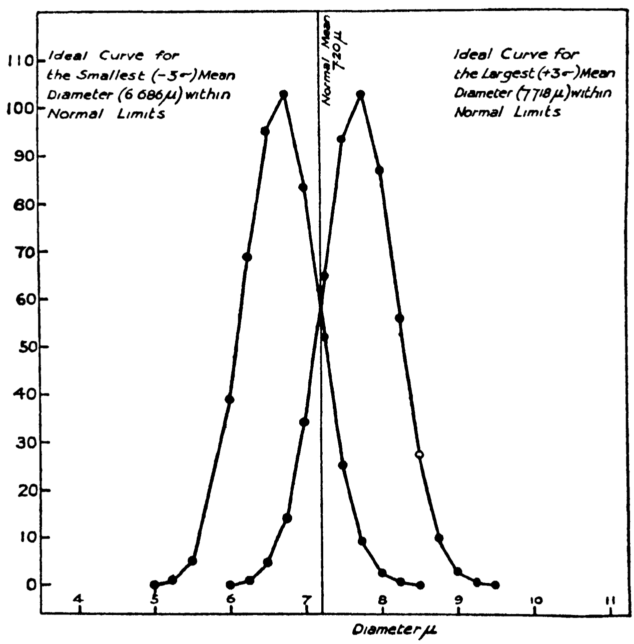

FIG. 312. Size-distribution curves of normal erythrocytes showing the extreme range for smallest and largest mean diameters (500 cells). The curve for average normal individuals is midway between these two. (PRICE JONES.)

one-third of this in thickness. In the dried, fixed film they are, owing to shrinkage, on the average only about 7·2 μ in diameter, extreme values being between about 6·7 and 7·7 μ. The actual size-distribution in normal people is shown in Fig. 312, which illustrates the narrow range of variation in size.†

* RIMINGTON. *Ergebn. d. Physiol.*, 1933, **35**, 712.

† PRICE JONES. *J. Path. and Bact.*, 1929, **32** 479.

The *average* size of the red cells can be determined in a film, fresh or dried, by a diffraction method,* using an instrument called an eriometer or halometer.

The red corpuscles are not of rigidly fixed size when in the body. The circumstance which is chiefly responsible for change of size in the body is alteration in the reaction of the blood plasma; reduction of the alkalinity of the plasma causes swelling of the corpuscles, and *vice versâ.* Hence it follows that the corpuscular volume is slightly greater in venous than in arterial blood.

The colour of a single corpuscle when viewed under the microscope is yellow, the red colour being apparent only when larger numbers are seen together. The red corpuscles form about 50 per cent. of the total mass of the blood. They are soft, flexible and elastic, so that they can readily squeeze through apertures and canals narrower than themselves, without undergoing permanent distortion. When examined by the usual histological methods, or when viewed in the fresh state by dark-ground illumination, the normal erythrocyte appears quite structureless. Each red corpuscle has been described as consisting of a framework or stroma, composed chiefly of protein and lipide material, containing in its meshes a red colouring matter, hæmoglobin, to which is due the colour of the corpuscles and of the blood itself. No limiting envelope is histologically demonstrable, though a convenient view is that a monomolecular layer of some material—probably lipides, such as lecithin—exists at the surface.

In nearly all mammalia the red corpuscles are of the character described (Fig. 313). In the camel they are oval in shape, but otherwise resemble the corpuscles of other mammals. In all other classes of vertebrata the red corpuscles are oval, nucleated cells; the hæmoglobin is diffused through the protoplasm of the cell body and does not extend to the nucleus. During the early part of fœtal life the corpuscles of mammals are also nucleated, but in the adult condition the erythrocytes, except under abnormal conditions, lose all traces of the nucleus before entering the blood stream. The small size and great number of the red corpuscles determine that a very large area of surface of red corpuscles is exposed to the plasma. This great extent of surface is of importance in facilitating the exchange of material, especially oxygen, between the corpuscle and the surrounding plasma.

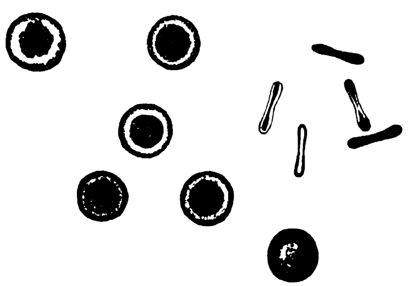

FIG. 313. Human Red Corpuscles. On the right of the figure the corpuscles are seen on edge. (SWALE VINCENT.)

The volume of each corpuscle has been estimated as about 90 μ^3 (= ·000000090 c.mm.), and its surface as 000128 mm.^{2}, so that the total surface of red corpuscles in the blood of a man weighing about 70 kilos (assuming his total blood as $\frac{1}{13}$ of the body weight) would be about 3000 sq. metres, or 1500 times the surface of the body itself.

Burker has shown that in various species there is a close relation between the surface area of the red cell and its hæmoglobin content, the ratio being constant at about 32×10^{-14} g. of hæmoglobin per square μ of corpuscle surface.

OSMOTIC RELATIONSHIPS OF THE RED CORPUSCLE. If the blood plasma be concentrated by evaporation or by the addition of neutral

* PIJPER, *S. Afr. Med. Rec.*, September 27th, 1919; ALLEN and PONDER, *J. Physiol.*, 1928, **66**, 37.

salts, its osmotic pressure rises, and water diffuses from the corpuscles into the plasma to equalise the osmotic pressure within and without the corpuscle. The latter therefore becomes wrinkled or *crenated*. On the other hand, dilution of the plasma diminishes its osmotic pressure to below that of the corpuscles, and water therefore passes into the latter, which swell up and become spherical; and, if the plasma be made sufficiently dilute, the corpuscles liberate the hæmoglobin they contain, leaving the colourless swollen *stromata* floating in the plasma. At the same time the blood becomes transparent and darker in colour, and is called "laked" or hæmolysed blood. This is called *hæmolysis*. In partial hæmolysis the corpuscles do not all burst; microscopical examination of the process with dark-ground illumination, shows that many corpuscles merely swell to a great size, the "ghosts" which remain after hæmolysis being nearly invisible to ordinary observation. They become more visible, and return to their former size, if the solution is made isotonic again. We may perhaps attribute the ultimate escape of the contents to the fact that the cell contains insufficient lipide to form a continuous layer over so large a surface.

Blood may be laked by any of the following means:—

(*a*) Addition of a small amount of ether or other lipide solvent.

(*b*) Free dilution with water, or dialysis.

(*c*) Alternate freezing and thawing of the blood.

(*d*) Addition of bile salts or of saponin.

(*e*) The action of foreign blood serum or of various hæmolysins whose nature we shall discuss later

The erythrocytes of different individuals, especially in certain pathological states, exhibit different amounts of resistance to hæmolysis. As a measure of the "fragility" or ease with which hæmolysis may be induced by hypotonic solutions, we may plot a curve showing per cent. hæmolysis at different concentrations of NaCl * and find the concentration at which 50 per cent. hæmolysis occurs. In normal blood this is at 0·334 to 0·398 per cent. NaCl: in hæmolytic jaundice it may be as high as 0·7 to 0·8 per cent.; after splenectomy fragility is reduced to 0·25 to 0·35 per cent. Cells of venous blood are slightly more fragile than those of arterial blood, and in general an increase in the ratio thickness/diameter of the cells increases fragility. Fragility is increased by change of *p*H above or below 8·1 and by cold.† The corpuscles of normal mammalian blood in presence of air, neither gain nor lose volume in a solution containing 0·9 per cent. sodium chloride. The osmotic pressure as determined by the freezing-point of such a solution is identical with that of the blood, which in most mammals has a freezing-point of about — 0·53° C. When the CO_2 is prevented from escaping from human blood, its osmotic pressure is nearer to that of 0·95 per cent. NaCl (men), or 0·93 per cent. NaCl (women). The salt solution which is normal for frogs' blood contains only 0·725 per cent. sodium chloride.

The behaviour of the red corpuscle, when immersed in various solutions, shows that its external layer is impermeable to most cations and neutral salts as well as to cane sugar and glucose. We may therefore make isotonic solutions with sodium chloride, sodium sulphate, potassium nitrate, or cane sugar. On the other hand, a solution of urea behaves towards the corpuscles like distilled water. If some red corpuscles be added to a 1 per cent. solution of urea in normal salt solution, they neither shrink nor swell, but if added to plain aqueous urea solutions of any strength the corpuscles

* CREED. *J. Path. and Bact.*, 1938, **46**, 331.
† DACIE and VAUGHAN. *Ibid.*, 1938, **46**, 341.

will be laked. There are many substances to which the corpuscles are permeable, *e.g.* alcohol, chloroform, ether, &c. In their permeability the corpuscles resemble most other vegetable and animal cells in permitting the passage of those substances that are soluble in lipides, which are invariable constituents of all living cells. According to Overton the external limiting pellicle of the red corpuscles, as in most living cells, is formed by a lecithin-cholesterol compound, whose solvent power determines the permeability of the cell by foreign substances.

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CHEMISTRY OF THE RED BLOOD CORPUSCLES

The chief constituent of the red cells is the chromoprotein, hæmoglobin, which is present chiefly as a potassium salt. It is to this substance that the red colour of blood is due.

The blood corpuscles contain a greater percentage of solids than any soft tissue of the body. The blood corpuscles have 37 per cent. solids, as against muscular tissue with 25 per cent. or nervous with 22 per cent. solids. Of these solids, 95 per cent. consist of hæmoglobin, so that the solution would have to contain at least 30 per cent. hæmoglobin, which is far beyond its solubility. Some form of combination therefore exists in the corpuscles, which keeps the hæmoglobin from separating out in crystalline form.

In investigations on the chemistry of the red cells it is usual first to remove the plasma by centrifugalisation and washing. The blood, which has been defibrinated, or prevented from clotting by the addition of a little sodium oxalate, is centrifuged until all the formed elements are thrown down as a solid cake at the bottom of the tube. The sediment is then mixed with normal saline fluid and again centrifuged, and this process repeated twice in order to wash away adherent plasma or serum. After the final washing the paste of corpuscles can be laked ; for this, two volumes of distilled water saturated with ether are added to one volume of caked corpuscles. The fluid is once more centrifuged in order to throw down white blood corpuscles. A 1 per cent. solution of acid sodium sulphate is now added drop by drop until the solution acquires the opaque appearance presented by ordinary blood. On centrifuging, the stromata are thrown down and can be collected and washed with distilled water several times on the centrifuge. Removal of stromata from laked cell solutions can be effected also by shaking for an hour or two with asbestos pulp and then removing this, with adherent stromata, by centrifugalisation. The stroma protein (a nucleo-protein) forms only about 4 per cent. of the total solids of the corpuscle. The cells also contain about 0·3 per cent. lipides, which is all in the stroma. About 10 to 15 per cent. of the dry weight of stroma material consists of lipides, of which 60 per cent. is phospholipide (half of it cephalin), 30 per cent. free cholesterol and 10 per cent. cholesteryl esters and fats.*

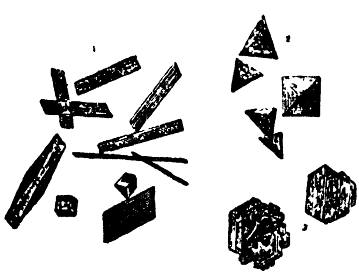


FIG. 314. Crystals of Oxyhæmoglobin. 1. From rat. 2. From guinea-pig. 3. From squirrel.

From a laked solution of corpuscles, oxyhæmoglobin can be obtained in a crystalline form with varying readiness according to the animal from which the blood is derived. Thus in the case of the rat, the guinea-pig, the dog and the horse, it is sufficient merely to cool the laked blood, preferably in a freezing mixture to about – 10° C., in order to obtain a large crop of hæmoglobin crystals. Crystallisation is facilitated by the addition of 25 per cent. of alcohol to the mixture, but this partly denatures the hæmoglobin. To avoid this, the following simple method depends on the fact that oxyhæmoglobin is less soluble than reduced hæmoglobin. Washed horse corpuscles are dialysed in collodion sacs against the osmotic pressure of the hæmoglobin. When fully laked,

* ERIKSON, *et al.* *J. Biol. Chem.*, 1938, **122**, 515.

débris is removed by centrifugalisation, and then the cooled solution well oxygenated, when oxyhæmaglobin crystals separate out. These can be collected, suspended in distilled water, and reduced *in vacuo*, when they dissolve; on re-oxygenation the solution again crystallises.*

Properties of Hæmoglobin. The crystals thus obtained are as a rule microscopic in size. Most animals yield an oxyhæmoglobin which crystallises in rhombic prisms or needles belonging to the rhombic system, though there are exceptions to this (Fig. 314). The solubility of the crystals differs according to the animal from which they have been derived, and is in direct proportion to the difficulty with which the crystals are obtained. They are more soluble in alkalies than in water and contain carbon, hydrogen, nitrogen, oxygen, sulphur and iron. The chief differences between various animals appears to have relation to the sulphur, and to the varying avidity with which the hæmoglobins combine with oxygen.† All specimens are alike in containing a constant proportion of iron, viz. 0·335 per cent.

Hæmoglobin readily combines with free oxygen to form oxyhæmoglobin, and it is found that for each atom of iron two atoms of oxygen can be taken up, *i.e.* 400 c.c. of oxygen per gramme of iron present in hæmoglobin. This corresponds to an oxygen capacity of about 1·32 c.c. oxygen per gramme of hæmoglobin, if we take 17,000 as the molecular weight for one atom of iron. The figure usually taken is 1·34 c.c. O_2 per g. Hb, but it is so difficult to prepare hæmoglobin pure that this figure is provisional. Oxyhæmoglobin is thus a compound, in definite proportions, of oxygen and hæmoglobin. It can be easily dissociated and is split up by such simple means as exposure to a vacuum (*v.* Chapter XXXIX); the fluid then gives off oxygen and the colour changes from a scarlet to a dull bluish red. The same change can be effected by treating a solution of oxyhæmoglobin with reducing agents such as hydrazine, sodium hydrosulphite ($Na_2S_2O_4$) or ammonium sulphide. The oxygen in oxyhæmoglobin can be replaced by equivalent quantities of some other gases. Thus if carbon monoxide be led through a solution of oxyhæmoglobin, oxygen is given off, and its place is taken by an equal volume of carbon monoxide with the formation of a more stable compound, carbon monoxide hæmoglobin. This body is dissociated only with extreme slowness and is unaffected by the addition of reducing agents. By using special precautions to prevent oxidation of the gas, the carbon monoxide can be replaced in this compound by nitric oxide, NO. We have therefore a series of three compounds which can be arranged in order of stability, thus:

NO-hæmoglobin.
CO-hæmoglobin.
O_2-hæmoglobin.

The poisonous properties of carbon monoxide are chiefly due to its power of turning out the oxygen from the oxyhæmoglobin, thus depriving the tissues of the oxygen which is normally carried to them by the red corpuscles.

Another compound of interest is *sulphæmoglobin* which is formed by the addition of H_2S to reduced hæmoglobin. Sulphæmoglobin is formed in the body when excessive intestinal putrefaction leading to formation of H_2S occurs, especially if certain drugs such as sulphonamide are being administered. The resulting condition of sulphæmoglobinæmia may be dangerous.

Hæmoglobin and its derivatives give well-marked absorption spectra. Thus dilute solutions of oxyhæmoglobin show two well-marked absorption bands between Fraunhofer's lines D and E. The centre of the band nearest

* Dudley and Lovatt Evans. *Bioch. J.*, 1921, **15**, 487.
† McCarthy. *J. Physiol.*, 1935, **86**, 77.

to D corresponds to λ 579, and is often spoken of as the α band, while the second band, the one next to E, called the β band, is broader, has less sharply defined edges, and its centre corresponds approximately to λ 542. On concentrating the solution or using thicker layers, a point is reached at which the two bands fuse into one, and with a still stronger solution the whole of the spectrum is absorbed with the exception of the red end.

If a reducing agent be added to the solution of oxyhæmoglobin, the two bands disappear and their place is taken by a more diffuse band lying midway between the two (Fig. 316), its centre corresponding to λ 559. This is the absorption spectrum of hæmoglobin (reduced hæmoglobin).

The spectrum of carboxyhæmoglobin is very similar to that of oxyhæmoglobin, the α band being slightly ($\lambda = 570$ mμ) nearer to the violet end. In a mixture of oxy- and carboxy-hæmoglobin, the shift of the α band is proportional to the fraction of the hæmoglobin present as the carboxy-compound, a fact which has been utilised by Hartridge for the determination of carboxyhæmoglobin by means of his reversion-spectroscope.* It is of a brighter red than oxyhæmoglobin. The fact that CO-hæmoglobin is not altered by reducing agents can be shown by adding sodium hydrosulphite to CO-hæmoglobin, when no change is observed. The amount of CO present in the blood may be estimated chemically by expelling all the blood gases by acidified ferricyanide solution, and subsequent analysis of the gases by the Van Slyke apparatus.†

All these derivatives of hæmoglobin, besides their absorption bands in the visible spectrum, have characteristic absorption of light in the ultra-violet spectrum. In the case of oxyhæmoglobin this absorption causes a band (Soret's band) which occupies the greater part of the spectral region between Fraunhofer's lines G and H with its centre at $\lambda = 415$ mμ. In reduced hæmoglobin this band is displaced towards the visible part of the spectrum.

Another compound of hæmoglobin with oxygen is *methæmoglobin.* This is not normally present in the body. It may be prepared by the addition of a ferricyanide, permanganate, or certain other oxidising agents to solutions of oxyhæmoglobin. It is a chocolate-brown substance, crystallisable, and gives an absorption band in the red between Fraunhofer's lines C and D ($\lambda = 634$mμ). It is unaltered by exposure to a vacuum. It contains no available oxygen, but may contain a hydroxyl group. Its iron is in the ferric state.‡ Hydrosulphite reduces this to the ferrous state, giving reduced hæmoglobin, from which, by shaking with air, oxyhæmoglobin can again be obtained. When methæmoglobin is formed from oxyhæmoglobin the whole of the loosely bound oxygen of the latter is given off, a fact which is utilised for the estimation of the oxygen held in combination in the blood.

The formation of methæmoglobin is shown in the approximate equation :

$$HbO_2 + K_3Fe(CN)_6 + KOH \rightarrow HbOH + K_4Fe(CN)_6 + O_2$$

in which it is seen that the hæmoglobin molecule is converted into methæmoglobin by change of the iron to the ferric state, the ferricyanide being simultaneously reduced to ferrocyanide.

Another derivative related to methæmoglobin is cyanhæmoglobin. It is formed by adding a cyanide to methæmoglobin.

Derivatives of Hæmoglobin. Hæmoglobin is a compound of an iron-containing coloured group, called *hæm,* with a protein which varies somewhat in different animals. The prosthetic group is identical in

* Hartridge. *J. Physiol.*, 1912, **44**, 1.

† Sendroy and Liu. *J. Biol. Chem.*, 1930, **89**, 133 ; Peters and Van Slyke. "Quantitative Clinical Chemistry," 1932, 328. (Baillière, Tindal and Cox.)

‡ Conant and Scott. *J. Biol. Chem.*, 1926, **69**, 575.

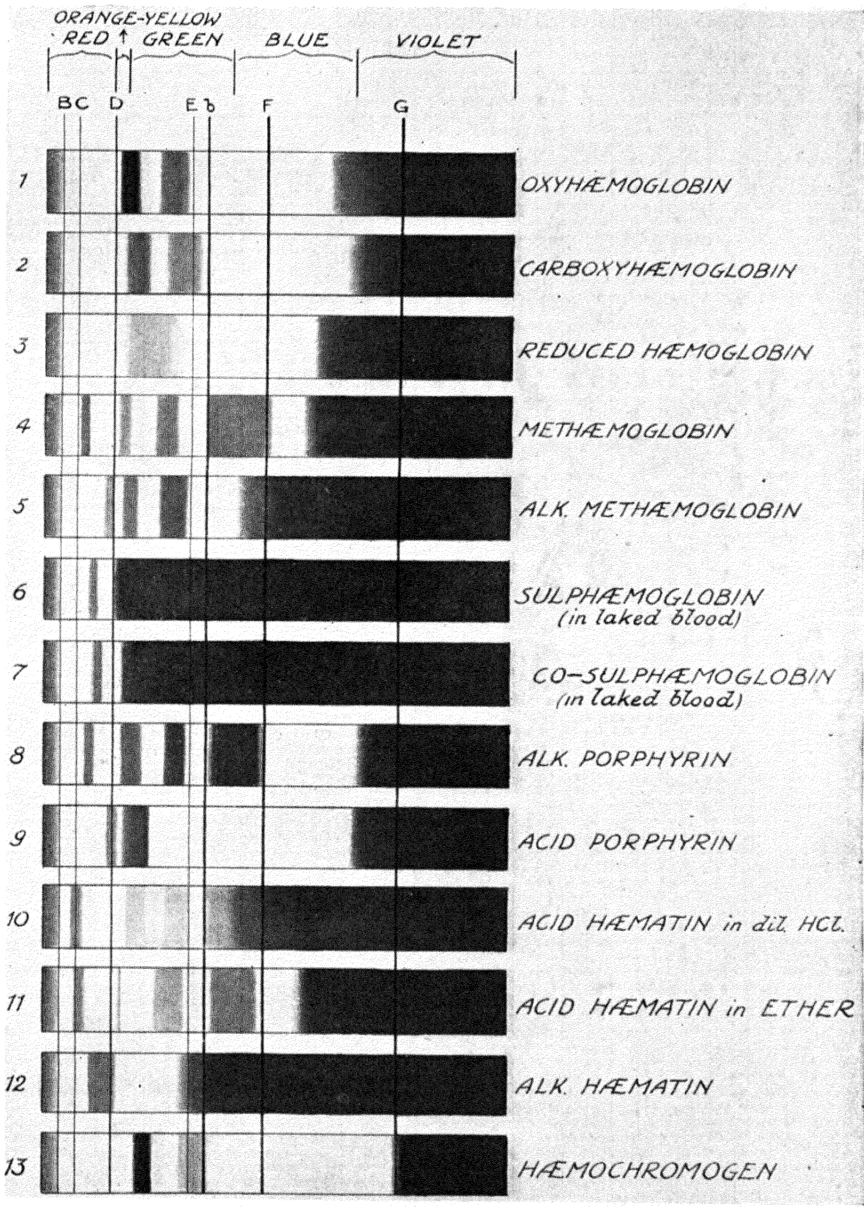


FIG. 316. Absorption spectra of hæmoglobin and its derivatives. (From HARRISON'S "Chemical Methods in Clinical Medicine." Churchill.)

[To face p. 530.

every case where it has been examined. A separation of the prosthetic group from the protein moiety can be effected with extreme ease and occurs whenever the hæmoglobin is treated with weak acids, with alkalies, or is heated above 70° C. The protein group is known as *globin.*

It is a strongly basic protein, classified with the histones. Unless prepared with great care the globin from blood is obtained in a denatured condition. Undenatured globin was prepared by R. Hill and Holden by breaking up oxyhæmoglobin solution with the smallest possible amount of dilute HCl at − 2° C., adding cold water, ether and kieselguhr and shaking well. It is then filtered and kept at − 2° overnight. Metaprotein is thrown down by adding ammonia, and is filtered off. The filtrate is freed from ether by vacuum treatment and the salts removed by dialysis.

Hæmoglobin yields about 94 per cent. of globin and about 4·5 per cent. of the chromogenic group, *hæmatin.* In order to obtain hæmatin in a pure condition, it is usual to start with the crystalline derivative *hæmin.* When some dried blood is heated with a crystal of common salt and glacial acetic acid on a slide, the residue contains reddish brown hæmin crystals (Fig. 315), and by a large-scale modification, hæmin may be prepared in quantity. It is a hydrochloride of hæmatin of the formula: $C_{34}H_{32}O_4N_4ClFe$ (Fischer). The iron is in the ferric state. By reduction in alkaline solution it yields the ferrous compound, *Hæm* ($C_{34}H_{32}O_4N_4Fe$). By dissolving hæmin in alkalies and adding excess of acid, hæmatin is precipitated. It is insoluble in water, alcohol or ether, but is slightly soluble in glacial acetic acid and in absolute alcohol. It is easily soluble in concentrated sulphuric acid, with decomposition, losing its iron and being transformed into *hæmatoporphyrin.* The formula of hæmatin is probably $C_{34}H_{32}O_4N_4Fe.OH$. Its compounds with acids and alkalies are spoken of as acid and alkaline hæmatin, and each gives a characteristic absorption spectrum (Fig. 316).

FIG. 315. Hæmin Crystals.

Hæmochromogen. In the presence of certain foreign matters, alkaline hæmatin, when treated with reducing agents, forms a substance known as hæmochromogen. The same change is observed when crude alkaline hæmatin, made by the action of alkalies on ordinary blood, is reduced by ammonium sulphide. These facts receive an explanation from the discovery by Anson and Mirsky * that the hæmochromogens are compounds of reduced hæmatin with one of many possible nitrogenous compounds. When made from blood, the nitrogenous compound is a denatured globin, but other proteins, or nicotine, hydrazine, pyridine or ammonia can take its place, to yield very similar hæmochromogens. When hæmochromogen is prepared from blood, the changes would be ·

Oxyhæmoglobin + alkali → Hæmatin + Globin (denatured)
Globin (den.) + Hæmatin [+ reducing agent] → Hæmochromogen.

When prepared from other compounds, *e.g.* pyridine, the hæmochromogen similarly consists of a compound of reduced hæmatin and pyridine. When reduced hæmoglobin is treated with alkali, the globin is denatured, but not split off, and hæmochromogen results at once. It is evident then that hæmochromogen differs from hæmoglobin essentially in the fact that its protein is

* ANSON and MIRSKY. *J. Physiol.*, 1925, **60**, 50.

denatured, and in existing only in the reduced form; when oxidised it yields alkaline hæmatin and the protein or other nitrogenous body is split off.

The hæmochromogens in solution have a cherry-red colour, and when sufficiently diluted show two well-marked absorption bands (Fig. 316) which are situated between D and E; that nearest to D has very sharply defined borders; the positions of the two absorption bands are λ 555 to 559 and λ 525 to 528. The band nearest D is given by 1 in 25,000 hæmochromogen solutions, so that it is an even more delicate test for blood than is the spectrum of oxyhæmoglobin itself. Hæmochromogen does not combine with oxygen, but does so with carbon monoxide. When CO-hæmoglobin is treated with alkali in the absence of oxygen, the CO-hæmochromogen formed contains exactly the same volume of CO in combination as the original CO-hæmoglobin.*

Cytochrome. Hæmochromogens are found widely distributed in almost all tissues of animals and plants, and have been given the name cytochromes by Keilin. They exist in the oxidised and reduced forms, the latter of which shows a characteristic spectrum (Fig. 317). In living tissues examined by the microspectroscope the formation of the reduced cytochrome can be witnessed when oxygen is excluded, and it has been shown that cytochrome plays an important part in the execution of oxidative changes in the cells.

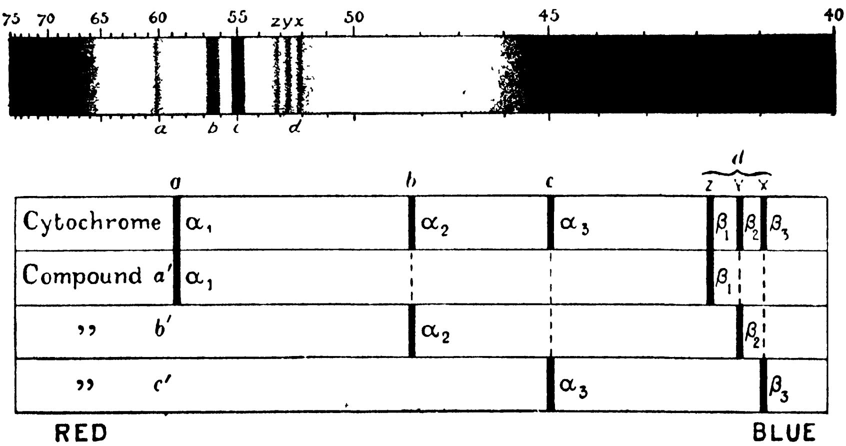


FIG. 317. *Above:* Absorption Spectrum of Cytochrome in Muscle of Bee. Reduced form as obtained by crushing tissue between two glass slips.
Below: Showing three of the separate spectra which together make up the above spectrum of mixed cytochrome. (Expanded scale) These components, and their bands, have now been re-named. Cytochrome *a'* now includes α and α_3, while *b'* and *c'* are called *b* and *c*. (After KEILIN.)

When oxygen is excluded, the tissues reduce it; when oxygen is readmitted it is oxidised again, and so it acts as an oxygen carrier. The amount of cytochrome in a tissue is closely related to the intensity of its respiration; moreover, when cyanide is added to the tissue, the cytochrome remains in the reduced state even when oxygen is allowed access to it, and at the same time the oxygen utilisation of the tissue is stopped. Tissue-cytochrome is a mixture of four cytochromes, called α, α_3, *b*, and *c* of different properties† (Fig. 317).

Hæmatoporphyrin. If hæmoglobin, hæmatin or hæmin be treated with a strong acid, it dissolves, forming a purple-red solution. On pouring this

* ANSON and MIRSKY. *J. Physiol.*, 1925, **60**, 65.
† KEILIN and HARTREE. *Proc. R. S.*, 1939, **127B**, 167.

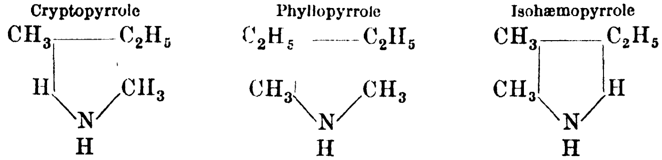
solution into a large quantity of water, hæmatoporphyrin is thrown down as a brown precipitate.

Hæmatoporphyrin is easily soluble in alkalies and somewhat less readily so in acids, forming alkaline and acid hæmatoporphyrin respectively. Solutions of alkaline hæmatoporphyrin show four absorption bands.

In the formation of hæmatoporphyrin from hæmatin, the iron of the latter has been split off by the action of the strong acid. The splitting off of iron occurs much more readily in the absence of oxygen. Porphyrins related to hæmatoporphyrin occur in minute quantities in normal urine and in larger quantities in certain toxic conditions, especially in poisoning by sulphonal, when the urine may have a red colour.

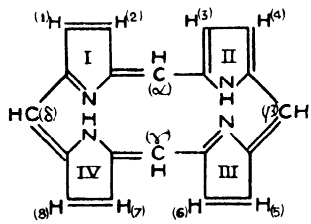
CHEMICAL RELATIONSHIPS OF HÆMOGLOBIN. If hæmin or hæmatoporphyrin be reduced by the action of hydriodic acid we get a mixture of substituted pyrroles formerly known as hæmopyrroles. The hæmopyrroles, according to Willstatter, are three in number and have the following formula :

Cryptopyrrole Phyllopyrrole Isohæmopyrrole



The same substances can be obtained from the chlorophyll of plants.

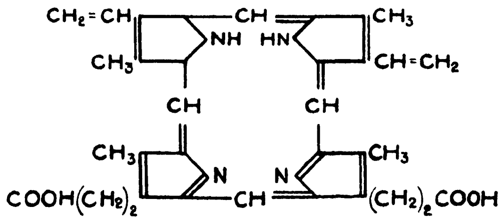
PORPHYRINS. Thus the same group forms the basis both of the substance which is responsible in the plant for the assimilation of carbon from carbon dioxide, and of the pigment which in the animal is the carrier of oxygen between the tissues and the surrounding medium. These and various other pigments found in animals and plants are derived from the chemical group of *porphyrins,* which consist of four substituted pyrrol rings. They are all derived from the parent substance *porphin* —



Porphin

The porphyrins of importance in the body are those known as I and III. These differ in their substituent groups. Hæmatoporphyrin may be taken as a type of III. Many porphyrins are known and some have been synthesised.

A possible structural formula for hæmatoporphyrin is (Fischer). : —



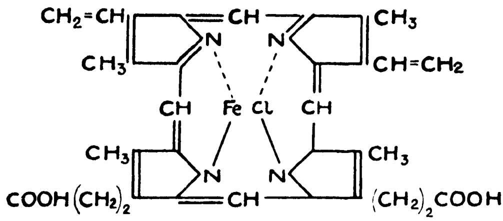
Derivatives of hæmatoporphyrin called uroporphyrins and coproporphyrins are found in urine and fæces respectively. They may belong to series I or III.

The relations of the chief simple porphyrins may be roughly shown as follows, designating the porphoryl group as Por :—

Protoporphyrin	Por · H_2
Uroporphyrin	Por : $(COOH)_6 . H_2$
Coproporphyrin	Por : $(COOH)_2H_6$

HÆMES. The porphyrins exist in various metallic derivatives called *metallo-porphyrins* ; those with iron form the group called *hæmes,* of which hæmatin and its derivatives are examples ; all the porphyrins form the corresponding iron-porphyrins when treated with ferric chloride and sodium acetate. Thus protoporphyrin gives hæmatin.

The following is a suggested formula for hæmin (H. Fischer and Zeile) * :



Of the iron porphyrins the following are important :—

Ferrous Compounds—

Reduced hæmatin (hæm)	Por : $Fe^{\cdot\cdot}$
Reduced hæmoglobin	(Globin) (Por : $Fe^{\cdot}$)
Oxyhæmoglobin	(Globin) (Por : Fe)O_2
Hæmochromogen	(Denatured globin) (Por : $Fe^{\cdot\cdot}$)

Ferric Compounds—

Hæmatin	Por : $Fe^{\cdot\cdot\cdot}$OH
Hæmin	Por : $Fe^{\cdot\cdot}$ Cl
Methæmoglobin	(Globin) (Por : $Fe^{\cdot\cdot\cdot}$OH)
Cyanhæmoglobin	(Globin) (Por : $Fe^{\cdot\cdot\cdot}$CN)

The power of loosely combining with O_2, CO and NO is confined to the ferrous compounds. Cyanhæmoglobin is, on the other hand, a CN derivative of a ferric compound, *e.g.*, of methæmoglobin and is not formed by addition of HCN to oxyhæmoglobin.

Metallo-porphyrins containing metals other than iron are also known to occur naturally, *e.g.* turacin, a feather pigment containing copper ; and compounds containing cobalt, nickel, zinc, &c. have been artificially prepared. Again, iron porphyrins in which the porphyrin part is different, *e.g.* the respiratory pigment chlorocruorin, found in certain worms, have recently been subjected to study.

THE SYNTHESIS OF THE BLOOD PIGMENTS. Many of the porphyrins can be made synthetically, and H. Fischer and his pupils succeeded in synthesising hæmatoporphyrin, hæmatin and hæmin. Given hæmatoporphyrin, moreover, Laidlaw † showed that the iron could easily be introduced by adding ferrous sulphate and ammonia : in presence of hydrazine hydrate hæmochromogen was formed, and from this hæmatin.

The pigment called turacin, occurring in the wing feathers of certain birds, was shown by Church to contain copper and to yield, on treatment with strong sulphuric acid, a substance indistinguishable from hæmatoporphyrin. Laidlaw also succeeded in synthesising this pigment by treating hæmatoporphyrin with ammoniacal copper solution, showing that it is a compound corresponding to hæmatin, in which the place of iron is taken by copper.

The combination of globin and hæmatin to form hæmoglobin was effected

* FISCHER and ZEILE. *Ann. d. Chem.*, 1929, 468, 98.
† LAIDLAW. *J. Physiol.*, 1904, 31, 464.

by R. Hill and Holden.* They prepared globin from oxyhæmoglobin without denaturation, as described above; the globin after purification was mixed with hæmatin in sodium carbonate, when methæmoglobin resulted. This on reduction gave reduced hæmoglobin, which on shaking with air gave oxyhæmoglobin, recognisable by its absorption spectrum. If denatured globin is employed, hæmochromogen is formed instead of methæmoglobin.

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THE LIFE-HISTORY OF THE RED BLOOD CORPUSCLES

BEFORE BIRTH. At a very early stage of development there arises from the mesoderm in various situations the primitive connective tissue, or *mesenchyme*, and it is in this tissue, or from its direct descendants, that both blood cells and vessels are developed.

The first appearance of blood cells is in mesenchyme tissue situated outside the embryo itself, in the *area opaca* of the yolk sac, and in the amnion and chorion. Here are formed the blood islands, groups of cells in which the peripheral mesenchyme cells form the endothelium of the vessels, an intercellular fluid, the plasma, and the more centrally placed cells, the *primitive blood cells*, all of which latter are nucleated cells which closely resemble the parent mesenchyme cells. In man the earliest vessels appear in this way on the under-surface of the yolk sac, and gradually extend over its surface, finally establishing connection with the similar vessels of the chorion. By the third week communication has been established between this system and another system of vessels, namely the blood vessels and heart, of the embryo itself. The vessels of the embryo are likewise developed from the mesenchyme, but with the difference that in these vessels the differentiation of the mesenchyme is into *endothelium* and plasma only, with practically no primitive blood cells. The first blood cells of the embryonic circulation, therefore, represent an invasion of primitive blood cells from the yolk sac vessels into the vascular system of the embryo, when connection has been established between the two systems.

The primitive blood cells undergo further differentiation, and from them are derived cells called *hæmocytoblasts*. These cells are the primitive stem cells from which, according to some authorities, all the cells of the blood are ultimately derived. In the formation of the red cells, these cells are converted into the immediate precursors, called *erythroblasts*, and from these, by the formation of hæmoglobin, into the fully formed red cells (Fig. 318).

ORIGIN AFTER BIRTH. In the fœtus the cells reach the circulation in an immature form and are nucleated, but soon after birth all the corpuscles are of the non-nucleated type. They are now formed in the red marrow of the bones, and can be shown to be derived from nucleated erythroblasts by a process of degeneration and solution of the nucleus. We have definite proof that a continual formation of red corpuscles is proceeding, throughout the whole of adult life. In an adult the total number of corpuscles remains approximately constant. By bleeding an animal we can diminish the total amount of corpuscles. The first effect of such a bleeding is that the fluid

* HILL and HOLDEN. *Bioch. J.*, 1926, **20**, 1326.

parts of the blood are made up, so that the volume of the blood is restored to normal and the blood therefore becomes relatively poor in corpuscles. In a few weeks, however, the corpuscular content of the blood is found to be once more normal, showing that the loss of corpuscles has been followed by a compensatory regeneration. The fact that the pigments constantly leaving the body with the urine and fæces, namely, urobilin and stercobilin, are derived from bilirubin, which arises from hæmoglobin, indicates that constant

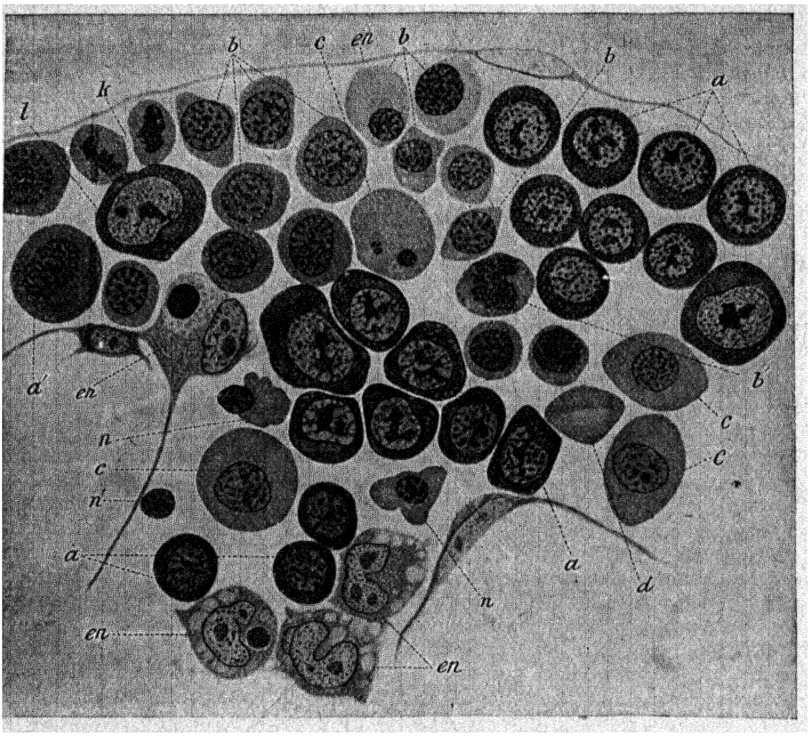


FIG. 318. Part of a Blood Vessel from the Yolk Sac of a Rabbit Embryo, showing the Changes which occur in the Formation of Erythrocytes. (From SCHAFER after MAXIMOW.)

a. Megaloblasts.
b. Normoblasts, changing into erythrocytes.
c. Erythroblasts, in which the nuclei are dividing.
d. An erythrocyte fully formed, but not yet disc-shaped.
en. Phagocytic endothelial cells.

destruction of red blood cells must be proceeding. Since the number of corpuscles remains unaltered, this loss must be made good by a continual regeneration of fresh red corpuscles.

That the marrow is involved in the process is shown by the fact that it is the only tissue of the body which undergoes an alteration in appearance when blood formation is reduced by starvation, or stimulated by such means as repeated bleeding or by destruction of corpuscles by the injection of toxic agents. In adult animals there may be three forms of bone marrow, yellow, gelatinous and red. By starvation, especially in birds, the amount of yellow marrow is increased, at the expense of the red; when the animal is fed again the yellow marrow is changed first to the gelatinous and then to the red form. In the yellow marrow there is abundance of fat cells, and the blood-forming

tissue is small in amount, and dormant. In adult mammals, the yellow marrow normally occupies the shafts of the long bones, and the red marrow the cancellous spaces in such bones as the vertebræ, ribs and epiphyses. After extensive blood destruction, the red marrow is found to have increased in extent and in many cases to have invaded the greater part of the shaft of the bone, taking the place of the yellow marrow. It is in the red marrow, therefore, that we must seek the precursors of the red blood corpuscles.

One important aspect of the subject of blood-formation is the nature of the blood vessels in the bone marrow. These are quite entire,* *i.e.* completely

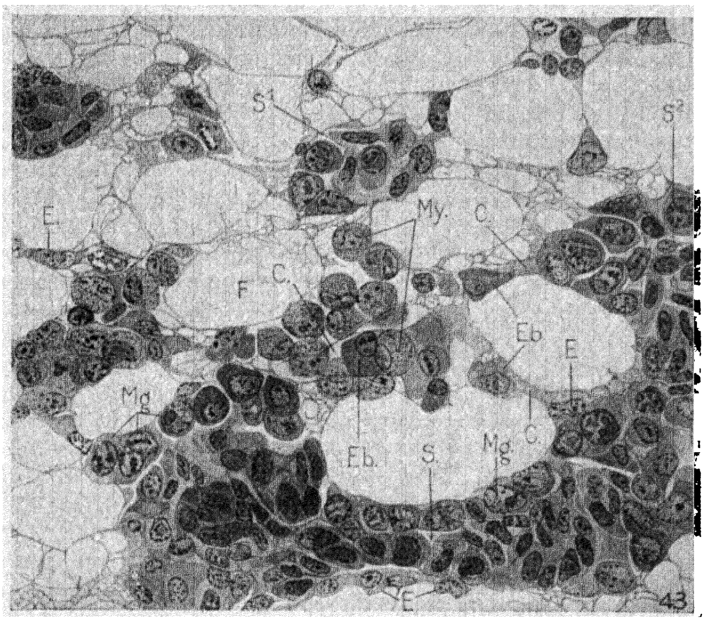


FIG. 319. Section of bone marrow of pigeon showing regeneration of red cells. Three sinuses, S, S^1, S^2, are seen filled with developing red cells. Along the inner wall are cells of the earlier phases—megaloblasts and early erythroblasts: note the difference in the depth of shade of cytoplasm as the hæmoglobin develops; in the centre of the sinuses are the older erythroblasts. In the middle of the field is a group of young myelocytes, ***outside*** a capillary, within which is a single erythroblast. Towards the right this capillary branches into two vessels, each containing a single erythroblast: one branch joins the sinus S^1, and the other joins sinus S^2.

C, capillary; E, endothelial cell; Eb, erythroblast; F, fat-cell; Mg, megaloblast; My, myelocytes; S, S^1, S^2, sinuses. (DOAN, CUNNINGHAM and SABIN. *Contrib. to Embryol.*, 1925, **83**, 165.)

lined by endothelium, and consist, in addition to arteries and veins, of a very rich plexus of sinusoids. But it is a fact of great importance that not all of these sinusoids are open at any one time, many of them being collapsed and admitting no passage of corpuscles through them. This is particularly evident in the gelatinous marrow.

The changes concerned in red-cell formation are most easily studied in birds in which the process has been checked by starvation. If such an animal is fed, it is found that many of the sinusoids become shut off as soon as blood formation commences, and it is in these closed vessels that the process of formation of the red cells occurs (Fig. 319); as soon as it is complete, the sinusoids open up, and the new cells are swept into the circulation.

* DRINKER, DRINKER and LUND. *Amer. J. Physiol.*, 1922 **62**, 1.

The red cells are formed from the endothelial cells of the closed sinusoid; these swell and divide and become so-called *megaloblasts*. These then become *erythroblasts* and then reticulocytes by change and loss of the nucleus, and end by becoming erythrocytes. In the process of maturation, hæmoglobin is formed, probably at the expense of the nucleus, which, however, is still retained in the erythrocytes of birds.

In mammals the process is very similar, except that *megaloblasts* are not normally seen. The *erythroblasts* are often to be seen in process of division, and the nucleated daughter-cells appear to undergo a process of karyolysis, the nucleus slowly disappearing, sometimes with preliminary fragmentation, as the hæmoglobin increases in the developing cell. When blood formation is quickened as the result of previous destruction or loss, some immature and still nucleated blood discs may make their way into the circulation and be found in the blood, where they are spoken of as *normoblasts*. Other young, though non-nucleated, cells (*reticulocytes*) can also be distinguished by the fact that they stain with cresyl blue, while the older erythrocytes do not. In the blood of normal adults only about 1 per cent. of the red cells are reticulocytes. It takes about seven days for the passage from megaloblast to reticulocyte, and a further two days for conversion to the erythrocytes.

Examination of the bone marrow in the human subject is carried out by puncture of the sternum and making a smear preparation of the extracted marrow.

CONDITIONS FOR RED CELL FORMATION. The fact that the rate of formation of new red cells keeps pace with the rate of their destruction, indicates that there must be some stimulus which acts appropriately on the red marrow. The stimulus is more effective in presence, or else is supplemented by the prevalence, of low oxygen pressures and *vice versâ*. Hence, the blood of animals exposed to atmospheres deficient in oxygen soon contains more corpuscles than when the animals breathe ordinary air, while in animals breathing oxygen-rich air the corpuscles are reduced in number.*

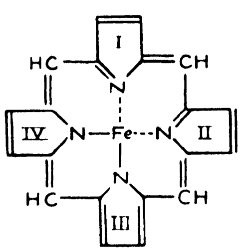
Some stimulus is also necessary to promote maturation. We do not know the exact nature of this stimulus, but it is certainly in part of a chemical nature and may emanate from the liver. For instance, Minot and Murphy † discovered that feeding with raw liver has a most beneficial effect in pernicious anæmia, in which, in spite of great hypertrophy of the red marrow, conversion of megaloblasts to erythroblasts is deficient, and Cohn ‡ found that extracts of liver having the same effect can be prepared. The administration is followed in four days by a sudden increase in the reticulocytes, which reach a maximum at about the eighth day. The active compound is called the *hæmatinic factor*. Highly active preparations, giving effects by injection, have been made, and it has been shown that the substance is probably an organic compound of relatively simple nature, which, however, is as yet uncertain. The most probable suggestion that it is a polypeptide containing an amino-hexose resembling glucosamine.§ A similar substance *ventriculin* has been prepared from hog's stomach, and there are indications that the substance in fact always originates in the stomach from which it is carried to, and stored in, the liver. The origin of the substance in the stomach has been supposed to be from an *extrinsic factor* present in certain foods, *e.g.* meat, yeast, rice-polishings, eggs (but not identified with any vitamin B component), and an *intrinsic factor*

* CAMPBELL. *J. Physiol.*, 1926, **62**, 211.
† MINOT and MURPHY. *J. Amer. Med. Assoc.*, 1926, **87**, 470.
‡ COHN, MINOT *et al.* *Journ. Biol. Chem.*, 1928, **77**, 325.
§ DAKIN and WEST. *J. Biol. Chem.*, 1935, **109**, 489.

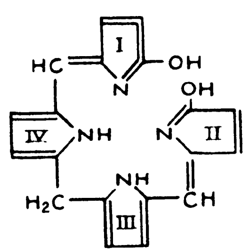
(hæmopoietin), possibly an enzyme, formed in the stomach itself.* In pernicious anæmia the intrinsic factor is lacking. If meat be partially digested in a normal stomach, or *in vitro* with fresh gastric juice, and then transferred to the stomach of a pernicious anæmia patient, the presence of the complete "*hæmatinic*" factor is revealed by the presence, after a time, in the blood of newly formed reticulocytes. It is a significant fact that pernicious anæmia is always associated with diminished gastric juice formation. The extrinsic factor is thermostable and soluble in 80 per cent. alcohol, but its nature is unknown. It is probable that the hæmatinic factor present in dried hog's stomach is formed in the process of drying and preparation by the interaction of the two factors.

Iron is, of course, essential for new red cell formation, and it has been discovered that in rats this is more efficiently carried out if traces of copper are also present in the diet.† The copper does not affect the assimilation of iron, but accelerates the conversion of assimilated iron into hæmoglobin.‡ Copper is present in the blood in organic combination with protein.§ Riboflavin also accelerates hæmoglobin production.

Destruction of Red Cells. How long a corpuscle continues to exist in the circulating blood is not known with certainty, though there are indications that, in man, the average life of the corpuscle is about three weeks.‖ Sooner or later, however, every corpuscle undergoes disintegration, a process generally ushered in by the ingestion of the corpuscle by some phagocyte cell. Thus, in the hæmolymph glands and in the spleen, we find large reticulo-endothelial cells which have englobed red corpuscles and in which we can recognise pigment granules derived from their destruction; this process of destruction of red cells by macrophage cells has been seen to occur in tissue cultures under the microscope.¶ The chief places of disintegration of the hæmoglobin are certainly the liver and spleen, and in these organs the hæmoglobin is converted first into biliverdin and then into bilirubin, which is then excreted by the liver. The approximate relationship between hæm and bilirubin is shown thus in skeleton form :—



Hæm



Bilirubin

It will be noted that the ring structure of the porphyrin has been opened up between I. and II. The liver cells proper do not form bilirubin, but merely excrete the bilirubin which is formed in the various cells of the reticulo-endothelial system. In the destruction of the corpuscles, the hæmoglobin is dissociated first into its protein and chromogenic moieties ;

* Castle, Heath and Strauss. *Amer. J. Med. Sci.*, 1931, **182**, 741 ; West. *J. Amer. Med. Assoc.*, 1935, **105**, 432.

† Hart *et al.* *J. Biol. Chem.*, 1928, **77**, 797 ; 1929, **84**, 115 ; Orton, Underhill and Lewis, *J. Biol. Chem.*, 1932, **96**, 1 ; Sheldon, *Brit. Med. J.*, 1932, 869.

‡ Elvehjem and Sherman. *J. Biol. Chem.*, 1932, **98**, 309.

§ Mann and Keilin. *Proc. R.S.*, 1938, **126B**, 303.

‖ Escobar and Baldwin. *Amer. J. Physiol.*, 1934, **107**, 249.

¶ Rich. *Bull. Johns Hopkins Hosp.*, 1924, **35**, 415.

the hæmatin then loses its iron and is converted into bile pigment. The iron remains in combination in the cells of the liver, spleen, &c., in the form of brown granules of a substance called *hæmosiderin*. This contains about 17 per cent. of iron, probably as a colloidal ferric oxide adsorbed on an organic substratum * It is probably utilised for the formation of the fresh hæmoglobin in the bone marrow. A section of normal liver immersed in potassium ferrocyanide and then in acid alcohol shows the presence of iron by blue granules in the liver cells. The amount of iron which can be demonstrated in the liver in this way is enormously increased in any condition which augments the rate of blood destruction, *e.g* in pernicious anæmia or by the action of poisons which cause extensive hæmolysis.

The porphyrin portion of the hæmoglobin molecule is probably synthesised in the body, though it is possible that the bilirubin portion as well as the iron-containing substance may be re-utilised for the formation of hæmoglobin, if bile is led away by a fistula, a condition of anæmia results, but may be cured by giving bile or hæmoglobin by the mouth.

That the liver is by no means the sole seat of bile pigment formation is shown by the results of experiments by Whipple, F. C. Mann and their colleagues. The latter find that the liver can be completely removed from dogs, provided sufficient glucose is given to prevent the occurrence of hypoglycæmic symptoms; ten hours or less after removal of the liver definite jaundice develops, and the urine, blood plasma and adipose tissue are stained with bilirubin. The jaundice is increased if hæmolysed blood is injected.

When hæmoglobin is set free into the circulation by hæmolysis, or is injected, it is removed in three ways: by excretion in the urine, by absorption by reticulo-endothelial cells, to form bilirubin and hæmosiderin, and by breakdown in the blood stream forming methæmalbumin, an analogue of methæmoglobin,† This is eliminated by the liver and finds its way into the fæces as porphyrin III.

Bilirubin is present in small amount in normal blood plasma, but in jaundice the amount is largely increased. Its presence can be demonstrated spectroscopically, or by the *van den Bergh reaction*. In this test, the serum is precipitated with alcohol and the filtrate added to a freshly prepared mixture of sulphanilic acid, hydrochloric acid and sodium nitrite, when a violet-red colour is produced.

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THE WHITE BLOOD CORPUSCLES

These are of various kinds, some of which, when kept warm, exhibit amœboid movements. Ehrlich showed that some corpuscles contained granules which were stained by acid dyes such as eosin, and these corpuscles he

* Cook. *J. Biol. Chem.*, 1929, **82**, 595.
† Fairley. *B.M.J.*, 1940, *ii.*, 213.

called *eosinophile*. Others, the *basophile* cells, were stainable with basic dyes, while a third type were only lightly stainable, and were called *neutrophile* leucocytes. The white corpuscles are classified as shown below (Fig. 320), the figures expressing the average results of what is called a *differential cell count*. This shows, out of a total of 100 white cells, how many of each variety present.

(1) GRANULOCYTES.
  (*a*) Neutrophile. Approximate average, 68 to 70 per cent.
  (*b*) Eosinophile. Average, 3 per cent.
  (*c*) Basophile (mast cells) Average, 0·5 per cent.

(2) MONOCYTES.
  (*a*) Large mononuclears. } Average, 4
  (*b*) Transitional. } per cent.

(3) LYMPHOCYTES. Average, 23 to 25 per cent.

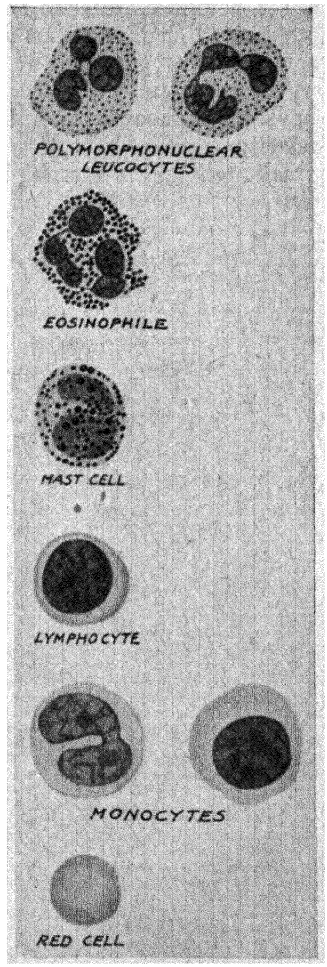

FIG. 320. Various forms of Blood Corpuscles.

The various white cells have the following characters:

(1) GRANULOCYTES.—(*a*) *The Neutrophile Cells*, or *polymorphonuclear* leucocytes. These present a lobed nucleus, and their protoplasm contains abundant fine neutrophile granules which stain with Ehrlich's triacid and with the May-Giemsa stains. The cytoplasm contains proteolytic enzymes capable of causing autolysis (hence the liquefaction of pus on standing). These cells are actively amœboid and phagocytic, and their numbers rapidly increase in pyogenic infections, in which they form pus cells.

(*b*) *The Eosinophile Cells*. The nucleus is generally single, but is often crescent-shaped or reniform. The protoplasm is crammed with large, discrete, highly refractive granules, which stain deeply with eosin and give micro-chemical reactions for iron as well as phosphorus. A large fraction of the histamine of the blood is present in these cells.* The eosinophile cells are not normally phagocytic.

(*c*) *The Basophile* (*or Mast*) *Cells*. These, which are somewhat smaller than the polymorphonuclear cells, have a lobed or tri-lobed nucleus and present in their protoplasm a number of granules which stain deeply with basic dyes. In normal blood the basophile cells are rare, though they appear, sometimes in large numbers, in disease, and are normally present in the blood of some of the lower animals.

(2) MONOCYTES.—These are large vacuolated cells with a single nucleus, and with fairly abundant faintly granular cytoplasm. The large mononuclears are rare cells in normal blood, but they are of considerable importance. They have the property of ingesting foreign particles of almost any sort, and for this reason are able to take up certain intravitam dyes, such as pyrrol or trypan blue, or granules of Indian ink or carmine.

The *transitional cells* are more numerous, as a rule, than the large mononuclears, forming about 4 per cent. of the total whites. Their general structure resembles that of the monocytes, but they are smaller. Their nucleus is indented or slightly lobed, and the cytoplasm has fine granules which stain with azur.

* CODE. *J. Physiol.*, 1937, **90**, 349, 485.

(3) LYMPHOCYTES.—These abundant cells, smaller than the other mononucleated white cells, and of more uniform diameter (about 8 μ), have a round nucleus and only a small amount of cytoplasm, which has very few granules, but such as it has are coarse and stain with azur.

The leucocytes as a whole undergo considerable variations in number according to the physiological state of the animal.

FORMATION OF THE LEUCOCYTES. The white cells are continually being lost and replaced. The normal life of a leucocyte is variously estimated at four days or upwards, but many die prematurely. The question as to the origin of each kind of leucocyte cannot be regarded as settled. According to Maximow and others, all the cells, red and white, are derived from one kind of cell called a *hæmocytoblast.* This opinion seems now to be losing ground, in favour of the view of Sabin, according to which erythrocytes and clasmatocytes are of endothelial origin, while the granulocytes, lymphocytes and monocytes arise from a common parent cell. This parent, or *reticular cell* is converted to a *primitive white cell,* examples of which are found in lymph glands, spleen and bone marrow. The subsequent history of these cells differs according to their situation, the two chief sites being lymph gland and marrow.

Lymphocytes are derived from the reticular cells of the lymphatic glands and the lymph nodules surrounding mucous cavities. These lymphatic nodules present towards their centre a clearer zone, consisting of lymphoblasts. By the division of these cells lymphocytes are formed, pushing towards the periphery of the nodule, where they make their way into the lymph sinus and are carried in large numbers (200 million hourly in the dog) by the lymph into the blood. Where they then go, or what their fate may be, is unknown.

In the red bone marrow granulocytes originate, outside the blood vessels, from the primitive white cells, which may be seen to be formed in abundance from the reticular cells during the gelatinous phase of the marrow. From these, *myeloblasts,* and then *myelocytes* of various types are formed, and divide actively, forming groups of cells. These contain a single rounded nucleus and a well-marked protoplasm which may contain granules, generally eosinophile in character but sometimes basophile. All intermediate states are to be found in the bone marrow between these myelocytes and the polymorphonuclear and eosinophile leucocytes. When mature, the granulocytes pass through the walls of the sinusoids into the blood stream. In leukæmia there may be myeloblasts in the blood as well as an increase either of eosinophile cells or of neutrophile cells, and either condition is associated with changes in the red bone marrow.

The monocytes are principally formed from reticular cells in the lymph glands and spleen, though exceptionally they, and also lymphocytes, may arise in the marrow. In certain abnormal states, clasmatocytes may also be present in the blood. We may therefore provisionally arrange the leucocytes of the blood according to their origin as follows :

(1) Small lymphocytes derived from lymphoid tissue.

(2) Monocytes, formed in the lymph glands and spleen, or exceptionally from reticulo-endothelial cells in other situations.

(3) Polymorphonuclear leucocytes formed in bone marrow.

(4) Eosinophile cells derived from similar cells in the bone marrow.

In certain diseases, the primitive white cells may develop along lines which are abnormal for the situation in which they occur; thus myeloblasts may appear in the spleen or even in lymph glands, and monocytes in the marrow.

FUNCTIONS OF THE LEUCOCYTES. The polymorphonuclear leucocytes present two phenomena, that of amœboid movement and that of ingesting foreign particles which may be presented to them. On account of this power of eating up foreign particles they are frequently spoken of as 'phagocytes.' Many unicellular organisms and the undifferentiated cells of many kinds of tissue behave similarly. All the phenomena connected with the process of *inflammation* in higher animals are directed to the assemblage of polymorphonuclear leucocytes at the spot which is the seat of injury or of infection, so that they may devour and remove either the injured tissue or the invading micro-organisms. This process therefore plays an important part in determining the immunity of any animal against infection; though in the higher animals it is assisted by a number of other phenomena directed towards the same end.

THE RETICULO-ENDOTHELIAL SYSTEM. In addition to the polymorphonuclear leucocytes, certain other cells have phagocytic properties. These are the monocytes of the blood, and certain widely dispersed tissue cells. All of these arise, like the blood cells, from the mesenchyme of the embryo. Of the mesenchyme cells, some become the *fibroblasts* of the connective tissues. A number, however, retain their embryonic developmental potentialities, and become converted into tissue histiocytes belonging to one of two types, called respectively the *free and the fixed histiocytes*. The *free histiocytes*, wandering cells, or macrophages, have phagocytic properties, and are to be found in the connective tissues. By a change in their granulation they may become the *mast cells*, familiar as inhabitants of the connective tissues. The *fixed histiocytes*, or 'resting wandering' cells, are to be found in large numbers in the serous membranes, especially in the omentum, and also as histiocytes which have taken up the position of an endothelial lining. This scattered system of specialised 'endothelial' cells is sometimes called the *reticulo-endothelial system*. Examples are the so-called 'endothelial cells' which form the incomplete lining of the sinusoids of the liver and spleen. In the *liver* the cells are known as the *Kupffer* or stellate cells. This widely dispersed system comprises cells of very differing function, shape and appearance, but they all possess in common the property of ingesting foreign particles of varying grades of size. Even relatively coarsely particulate matter is taken up by these cells, *e.g.* by the Kupffer cells of the liver. Thus, if Indian ink or carmine is injected intravenously, granules of the pigment are to be found shortly afterwards in the Kupffer cells, and in the similar cells of the spleen and marrow, as well as in the polymorphonuclear leucocytes and monocytes of the blood stream. All foreign colloidal matter, including colloidal dyes, *e.g.* pyrrol blue, are also taken up by the cells.

Phagocytosis is not merely of use in protecting the tissues from infection. Whenever any surplus or dead tissue has to be cleared away, whether as the result of injury or in the course of the metamorphosis of organs, the phagocytic cells of the tissues play an important part. Thus the absorption of the tail of the tadpole is effected in the same way by means of phagocytes. In mammals, including man, the moulding of the long bones which occurs in the process of growth is effected by continual and coincident processes of absorption and new formation of bone. The absorption is carried out by means of special phagocytes called osteoclasts.

The other functions, which have been ascribed to leucocytes, are unimportant as compared with their *rôle* as phagocytes, and are all of them questionable. Thus some authors ascribe to leucocytes a significant part in the taking up of fat from the intestine and its carriage into the lymphatic system. In the coagulation of the blood, the leucocytes have been supposed to act by the discharge of substances which may be precursors of thrombin. The leucocytes and lymphocytes are also believed to play a part in the formation of anti-toxins.

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THE BLOOD PLATELETS

In addition to red and white cells, appropriately fixed and stained blood films also present a number of granular bodies, most of which are about 2μ in diameter, and which tend to aggregate together in clumps. These are the platelets or thrombocytes. Their number usually ranges from 280,000 to 760,000 per cubic millimetre. Their shapes vary considerably. Their central part is more strongly refracting than the periphery and stains with basic dyes, but is not regarded as a nucleus (Figs. 321, 322).

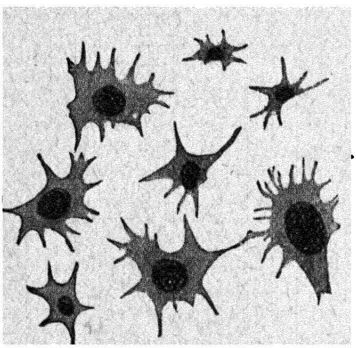

FIG. 321. Blood Platelets, highly magnified, showing the amœboid forms which they may assume. (After KOPSCH.)

Similar platelets are observed when the blood is received into normal salt solution; as the mixture clots, the filaments of fibrin can often be seen to radiate from a clump of disintegrated blood platelets as from a centre. They are enumerated by pricking the finger through a drop of 2·5 per cent. sodium citrate solution in normal saline. A drop is transferred, after further dilution with citrate-saline in marked vessels, to a slide, and the platelets and reds counted in appropriate fields. From the ratio between the two, and a separate red cell determination, the number per cubic millimetre are calculated. If the blood of an animal be defibrinated by repeated bleeding, whipping and returning to the veins of the animal, it will be found for the next few days to be quite free from blood platelets. Most histologists regard the platelets as derived by fragmentation from the cytoplasm of the giant cells, or megakaryocytes, of the bone marrow.

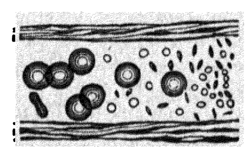

FIG. 322 Blood Corpuscles and Blood Platelets within a small Vein. (SCHAFER after OSLER.)

THE COAGULATION OF THE BLOOD

The subject of blood coagulation presents so many aspects on which there is disagreement that it will be necessary here to deal chiefly with what appear to be facts, and, since no other course is consistent both with brevity and with clarity, to treat the theory in a brief and dogmatic manner. The particular theory which will, for this purpose, be upheld, is that originally due to Morawitz.*

In the process of coagulation, while the red and white corpuscles remain almost or quite intact, the plasma becomes solid from the production in it of

* MORAWITZ. *Arch. klin. Med.*, 1904, **79**, 1, 25, 215. *Hofmeister's Beitr.*, 1904, **4**, 381.

a network of fibrin. The coagulation takes place most rapidly at body temperature and is greatly slowed by cold. There are many patterns of coagulometer by which the clotting time can be measured,* or graphically recorded.† Under constant conditions the time is constant for any individual. Under the ultramicroscope, the apparent network of fibrin is seen to have a crystalline structure.‡ The question of coagulation involves the consideration of the precursor of fibrin and of the conditions which determine its conversion into fibrin.

It will be clearer if the theory is outlined to begin with. The things in the blood which are concerned with clotting are, fibrinogen, prothrombin, platelets and calcium ions. Briefly, the precursor of fibrin which is present in the fresh plasma is the protein *fibrinogen.* It is converted into fibrin through the agency of an enzyme-like substance called *thrombin*, which is formed in the shed blood from a precursor called *prothrombin* § ; prothrombin exists in the normal plasma. The prothrombin is converted into thrombin by an activator, *thrombokinase* ; thrombokinase alone only slowly activates prothrombin, but in presence of calcium salts the activation is rapid. Thrombokinase is not present in circulating blood, but is formed from many kinds of tissue when these are damaged, and, among others,from the blood platelets. Hence, normal blood contains all the factors for clotting—platelets which form thrombokinase ; and prothrombin, which this activates in presence of the calcium salts, thus forming thrombin. The thrombin converts the fibrinogen into fibrin.

According to this theory, then, the prime mover in the series of changes terminating in coagulation is thrombokinase. We may now consider some of the facts.

Methods of Preventing Coagulation

It is evidently impossible directly to arrive at any conclusions during the few minutes which elapse before the appearance of the clot. We must therefore find some means of retarding coagulation, and then we can obtain a cell-free plasma by centrifugalisation, by which the corpuscles are thrown rapidly down to the bottom of the tube and the clear supernatant plasma can be drawn off and its coagulation studied.

(1) So long as the blood is in contact with the uninjured vessel it remains fluid. If the jugular vein of an animal be tied in two places, the blood contained between the ligatures will remain fluid, sometimes for days. If the tube of vein be hung up, the corpuscles sink to the bottom, and the plasma in the upper part of the tube can be poured from one vein to another without undergoing coagulation. On bringing it into contact with foreign substances, it undergoes coagulation.

(2) When an incision is made in the ordinary way into a blood vessel of a bird, the issuing blood clots very rapidly. The clotting is initiated by a substance contained in the tissues surrounding the vessels, because if the vessel be isolated and a perfectly clean glass cannula be inserted into it, care being taken not to bring the cannula in contact with any of the surrounding tissues, blood can be drawn off into a clean sterilised beaker and will remain unclotted for days.‖ Such blood can be centrifuged and the cell-free plasma used for experiment. The same does not apply to the mammal.

(3) Clotting can be excited even in the living vein by introducing into the blood any foreign solid, *e.g.* a thread, which is wetted by the blood. If the contact of shed blood

* Addis. *Q. J. Exp. Physiol.*, 1908, **7**, 314 ; Gibbs, *Q. J. Med.*, 1924, **17**, 312.

† Stoker. *J. Scientif. Inst.*, 1934, **11**, 315.

‡ Stubel. *Pflüger's Arch.*, 1914, **156**, 361 ; Howell, *Amer. J. Physiol.*, 1914, **35**, 143.

§ The names *thrombase* and *prothrombase* are sometimes substituted for thrombin and prothrombin.

‖ Delezenne. *Arch. de Physiol.*, 1897, **9** (Ser. 5), 333.

with such substances be prevented by receiving it into vessels coated with paraffin, and scrupulously free from dust, clotting may often be delayed for many hours.

(4) *Action of Cold.* If horses' blood, which always clots slowly, is cooled rapidly to between 0° C. and 1° C., it remains fluid for an indefinite time. The corpuscles sink, and the supernatant plasma can be decanted and even filtered.

(5) *Methods involving Mixture with Neutral Salts.* Blood is received into one-quarter its bulk of a 25 per cent. solution of magnesium sulphate, or into an equal volume of half-saturated sodium sulphate solution. The plasma obtained in either of these ways is known as salt plasma. Clotting can be induced by suitable treatment of the separated plasma.

(6) *Methods depending on Decalcification of the Blood.* Oxalate plasma is obtained by receiving blood into a solution of sodium oxalate so that the total blood contains 0·1 per cent. of the oxalate. Instead of oxalate we may use sodium fluoride (0·3 per cent.) or sodium citrate (0·35 per cent.).

(7) *Methods depending on the use of certain Substances of Animal Origin.* (*a*) *Peptone* plasma is obtained by injecting rapidly into the veins of a dog or cat in a fasting condition a solution of commercial peptone in the dose of 0 1 g. per kg. of the animal. This causes a state of shock which may last an hour or two. On drawing blood immediately after the fall of blood-pressure has taken place, it is found to be incoagulable. A number of animal extracts, such as extract of crayfish, of mussels, &c., act in a somewhat similar fashion. The addition of peptone to blood drawn into glass vessels does not inhibit clotting.

(*b*) *Leech Extract or Hirudin.* The bites made by leeches bleed for a considerable time, and it was shown by Haycraft * that this is due to the presence of an anti-coagulating substance in the buccal glands of the leech. This substance, which has the properties of an albumose, can be extracted from the anterior half of the leech by boiling. It will abolish the coagulability of the blood, either when injected into the blood stream or *in vitro*.

(*c*) *Heparin.* An anticoagulant substance, to which the name of heparin has been given, was first prepared from liver by Howell.† It is present also in muscle, lung, and various other tissues,‡ and may be obtained as a powder, of which 1 mg. will prevent the coagulation, at 0° C., of from 100 to 500 c.c. of blood. At room temperature 1 mg. will inhibit coagulation of 10 to 20 c.c of blood. A unit is 0 01 mg. It acts equally well *in vivo* or *in vitro*. Chemically, heparin is a polysulphuric ester of mucoitin, a substance related to chondroitin. Mucoitin is a condensation compound of glucuronic acid and acetylated glucosamine.§ Heparin is precipitated by protamine and although either substance alone will prevent coagulation, the two together are ineffective.

(8) *Methods depending on the use of Synthetic Organic Substances.* (*a*) *Chicago Blue, 6B,* injected intravenously or added to blood *in vitro* prevents coagulation.‖

An even better, and less strongly coloured dye is *chlorazol fast pink*, B.K.S.¶

(*b*) " Bayer 205 " (" Germanin ") ** or " Liquoid " (Roche), described as a sodium poly-anetholesulphonate.†† injected intravenously also cause incoagulability.

Factors in Coagulation

By centrifugalisation of blood the coagulation of which has been delayed or prevented in any of these ways, we obtain blood plasma. The conditions which will bring about coagulation in such plasmata are strikingly diverse. Thus, in cooled plasma a rise in temperature is sufficient, though if the cooled plasma be filtered several times at 0° C. through two thicknesses of filter-paper, it loses this spontaneous coagulability on warming. It can still be made to clot by the addition of certain substances, such as blood serum or the washings of a blood clot, and in some cases by the addition of tissue extracts. Oxalate plasma clots on addition of calcium salts. Sodium

* Haycraft. *Arch. f. exp. Path.*, 1884, **18**, 209.
† Howell. *Bull. Johns Hopk. Hosp.*, 1928, **42**, 199.
‡ Best. *B.M.J.*, 1938, *ii*, 977.
§ Jorpes and Bergstrom. *Z. f. physiol. Chem.*, 1936, **244**, 253.
‖ Rous, Gilding and Smith. *J. Exp. Med.*, 1930, **51**, 807.
¶ Huggett and Rowe. *J. Physiol.*, 1934, **80**, 82.
** Stuber and Lang. *Bioch. Ztschr.*, 1928, **194**, 204.
†† Fleisch *et al.* *Pflüger's Arch.*, 1932, **230**, 814.

sulphate plasma clots on dilution. Magnesium sulphate plasma after standing and centrifugalisation will not clot on dilution, but needs in addition the presence of some blood serum or some substance derived from blood serum. In both these cases tissue extracts have no influence.

CALCIUM IONS. We may take oxalate plasma as our type. It contains fibrinogen, prothrombin, a little thrombokinase, and an excess of oxalate. If calcium chloride be added drop by drop so as to be present in slight excess, and the plasma be then kept warm, it will clot. The place of calcium can be taken by strontium. Coagulation is also prevented by sodium fluoride, which precipitates calcium, and by sodium citrate, metaphosphate or pyrophosphate, which lead to the formation of a salt in which the calcium is in a complex anion. This shows that for calcium to exercise its function in clotting, it should be present in a form which yields calcium *ions*. The optimal $Ca^{\cdot\cdot}$ concentration is 5 mg./100 c.c., which is about that present in normal plasma, where about half the calcium is present in the ionic state, the remainder being colloidal and not ionisable. It is probable that calcium ions merely accelerate the activation of prothrombin by thrombokinase.*

FIBRINOGEN. Plasma contains a protein, *fibrinogen*, not present in the serum, and which must therefore be the precursor of fibrin. Fibrinogen is a globulin precipitated by half-saturation with sodium chloride. For coagulation experiments it is best made from bird's plasma † : the plasma is diluted ten times and neutralised by a few drops of 1 per cent. acetic acid. The fibrinogen precipitate is removed by centrifugalisation and dissolved in 0·5 per cent. sodium chloride. The solution still contains impurities. Fibrinogen is also soluble in dilute alkali, from which it is re-precipitated by acidification The solution of fibrinogen thus purified by repeating the precipitation and solution is perfectly clear and colourless; it coagulates between 56° and 60° C., whereas serum does not give any precipitate at 60° C.

THROMBIN. If a crude solution of fibrinogen be treated with a drop or two of calcium chloride, it rapidly clots. This is because it also contains prothrombin and thrombokinase In fact, evidence has been adduced ‡ that fibrinogen and prothrombin form a firm complex. If the fibrinogen has been thoroughly purified, however, calcium salts are found to have entirely lost their power of causing coagulation. Such a purified fibrinogen can still be made to clot by the addition of serum or of the washings of a blood clot, or of the watery extract of alcohol-coagulated serum. This power of serum to convert fibrinogen into fibrin is due to the presence in it of thrombin. The last change in the act of clotting is therefore the change from fibrinogen to fibrin, and this event is brought about by the intervention of thrombin. It cannot be at this stage of the process that the calcium salts exercise their influence, since thrombin will cause the coagulation of oxalate plasma. Thrombin is often regarded as an enzyme because it is active in minimal quantities. When in watery solution it is readily destroyed at 60° C. Rettger § and Howell || have, however, claimed that thrombin is not an enzyme, since if small quantities of thrombin solution be added to large quantities of fibrinogen, the amount of fibrin obtained is proportional to the amount of thrombin added. This is probably because some of the thrombin is adsorbed by the fibrin.¶

* MELLANBY and PRATT. *Proc. R S.* 1940, **128B**, 201.
† J. MELLANBY. *J. Physiol.*, **1917**, **51**, 396.
‡ MELLANBY. *J. Physiol.*, 1909, **38**, 28, 441.
§ RETTGER. *Amer. J. Physiol.*, 1909, **24**, 406.
|| HOWELL. *Amer. J. Physiol.*, 1910, **26**, 453.
¶ MELLANBY. *Proc. Roy. Soc.*, 1933, **113B**, 93.

Thrombin may be provisionally defined as a substance capable of producing clotting in calcium-free fibrinogen solutions. Accepting this definition, we can show that thrombin is absent from circulating blood. If blood be received direct from the vessels into absolute alcohol and the precipitate, after coagulation by alcohol, be extracted by water, the extract is found to contain no trace of thrombin. The same statement applies to fresh oxalate plasma. This contains the precursor of thrombin, which is called *prothrombin*, together with traces of another substance essential for the formation from it of thrombin, namely *thrombokinase*. Thrombin is best prepared by activation of prothrombin. Prothrombin is activated, at first slowly, then more rapidly, by thrombokinase, but in presence of calcium ions the rate of activation is greatly accelerated.* Hence, addition of calcium salts causes oxalate plasma to clot, and the serum squeezed from the clot is found to contain thrombin. In the early stages of coagulation, therefore, there is an actual formation of thrombin. Our next step must be to inquire into the precursors of the thrombin and the conditions of its formation.

PROTHROMBIN may be prepared by Mellanby's method †: Oxalated ox plasma is diluted with ten volumes of distilled water and brought to pH 5·3 by acetic acid. Fibrinogen and prothrombin are precipitated; the precipitate is treated with dilute calcium bicarbonate solution, which dissolves out the prothrombin while the fibrinogen remains in the precipitate. The supernatant fluid is a solution of prothrombin. Small amounts of the solution will not cause clotting of oxalated plasma. By reprecipitation at the isoelectric point, and drying the precipitate with acetone, a solid very rich in prothrombin is obtained. It keeps indefinitely, and when activated with calcium chloride and thrombokinase is so potent that 0 02 mg. of it will clot 1 c.c. of oxalated plasma in ten seconds; about 40 mg. of such a dry preparation is obtained from 100 c.c. of plasma. It has faintly acid properties, gives protein reactions, and is not dialysable. Apparently it is a globulin, and is insoluble in water, unless traces of alkali are added; the solution so formed is capable of withstanding a temperature of 100° C. for five minutes, though after this treatment it is only slowly activated by kinase and calcium salts. The common statement that thrombin is heat-stable owes its origin to experiments carried out with preparations containing prothrombin as well as thrombin; the former is heat-stable, the latter not.

The blood of new-born infants is poor in prothrombin, as is also the blood in obstructive jaundice. Administration of vitamin K, with bile salts, by mouth, restores clotting time of the blood to normal. It is therefore believed that the presence of vitamin K is essential to the formation of prothrombin, which is believed to be manufactured in the liver.

THROMBOKINASE. If oxalate plasma be cooled to 0° C. for two or three days, a scanty granular precipitate is produced. This consists largely of *débris* from platelets, which can be centrifuged off or separated by filtration through a Berkfeld filter. It is then found that the remaining plasma can no longer be made to coagulate by recalcification, although it still contains fibrinogen and prothrombin. If the precipitate be collected and treated with calcium chloride and the mixture added to the oxalate plasma, the latter clots. But if the precipitate is freed from adherent contamination, it will not clot pure fibrinogen, according to Mills. We must conclude that the precipitate, though itself not thrombin, will generate thrombin on treatment with prothrombin and calcium salts. It contains the activator, *thrombokinase* (sometimes called '*thromboplastic material*').

Oxalate plasma, which has been separated from the precipitate of

* J. MELLANBY. *Proc. Roy. Soc.*, 1931, **107B**, 271.
† J. MELLANBY. *J. Proc. Roy. Soc.*, 1930, **107B**.

platelets, can also be made to coagulate by the addition of calcium salts together with extracts of almost any animal tissues, and these therefore also contain thrombokinase. For use as thrombokinase it is usual to employ an extract made by grinding up fresh lung with sand and saline solution and collecting the clear fluid obtained by centrifugalisation.* These extracts, even on admixture with calcium, are without effect on pure solutions of fibrinogen, since they contain no prothrombin. But if the thrombokinase be treated with prothrombin solution and calcium salts, the mixture when added to oxalated plasma causes speedy clotting.

Thus far practically all workers on the subject are agreed. Coagulation of the blood is due finally to the interaction of thrombin and fibrinogen. Fibrinogen and prothrombin are present as such in the circulating plasma. Thrombin is not free in the circulating blood, but is produced or liberated after the blood has been shed. For the production of thrombin the presence of thrombokinase and of calcium ions is necessary.

It was shown by Howell that the phosphatid *cephalin* is an essential constituent of thrombokinase, in which it probably occurs in association with a protein. Similar substances are present in nearly all tissues, and are set free when the cells are injured. Separation of the blood platelets by cooling and filtration abolishes the spontaneous coagulability of any form of plasma by removing the thrombokinase.

Three factors are therefore necessary for the production of thrombin: first calcium ions; secondly thrombokinase, and thirdly prothrombin.

It is therefore concluded that when blood leaves the vessels there is a disintegration of the blood platelets, with the liberation of thrombokinase. This acts upon prothrombin in the presence of calcium ions and produces thrombin. By the intermediation of the thrombin, the fibrinogen also present in solution in the plasma is converted into fibrin. The changes occurring in shed blood and resulting in the production of a clot are therefore mainly concerned with the production of thrombin.

This view of the essential character of coagulation is borne out by observations on other forms of plasma, especially of that of birds' blood. This, when obtained with scrupulous cleanliness, so as to avoid any contamination with dust or with the tissues, remains permanently uncoagulable. In the plasma got by centrifuging the blood, no blood platelets are to be seen, and no precipitate is produced by exposure to a temperature of 0° C. We may say, therefore, that blood platelets with their contained thrombokinase have been removed from the plasma, and with them the property of spontaneous coagulability. Since it contains prothrombin as well as soluble calcium salts, it is only necessary to add thrombokinase solution, *i.e.* a tissue extract, in order to cause immediate clotting.

In every case the initiation of the act of clotting would seem to depend on the addition of thrombokinase to the plasma. In mammalian blood there is no appreciable disintegration of the red or white corpuscles when the blood leaves the vessels, and the first act in coagulation is probably the disintegration of the blood platelets, with the setting free of thrombokinase.

The part played by the platelets in coagulation is of great importance in maintaining the integrity of the vascular system. If an injury be made to the wall of a blood-vessel which is kept under observation by the microscope, it will be seen that the blood, as it flows past the injured spot, deposits blood platelets on the site of the puncture. These aggregate to form a white

* SEEGERS *et al.* *J. Biol. Chem.*, 1938, **126**, 91, give further details and also describe preparation of a highly active thrombin.

thrombus as a plug closely adherent to the wall of the vessel, and effectively preventing any escape of its contents. The white thrombus is almost entirely composed of agglutinated platelets, held together by fibrin. Later, by the growth of the adjacent endothelial cells, the thrombus is organised and covered with a layer of endothelium continuous with the rest of the vessel. The same process occurs when any part of the lining membrane of a large vessel is injured or when the blood is brought into contact with foreign matter, *e.g.*, if a glass or cellophane tube is inserted in the course of a blood-vessel. Thus, destruction of a patch of endothelium in a vein leads to the deposition of blood platelets over the patch and the formation of a 'white thrombus' adherent to the wall and tending to spread down stream.* Under healthy conditions the thrombus serves simply to cover the bare area in the wall of the vein, and is grown over later by endothelium, so restoring the integrity of the vessel wall. The formation of the white thrombi can be prevented by adequate injections of heparin (Best, *et al.*).

Citrated or oxalated blood can also be caused to clot by the addition of trypsin, and by certain snake venoms, which seem to act as thrombokinase. The Russell viper venom is a convenient source of thrombokinase for estimations of prothrombin.†

An important factor in the arrest of hæmorrhage is the increased coagulability of the blood, which clots more rapidly after a considerable fraction of the circulating blood has been withdrawn. Several causes contribute to this, but the most important is probably an increase in the fibrinogen content of the blood. We have seen that fibrinogen is manufactured in the liver, and it would appear that the rate at which the liver adds fibrinogen to the blood is accelerated after a large hæmorrhage. One factor in producing this effect is stated to be the presence of an increased amount of adrenaline in the circulating blood ; as soon as the arterial pressure begins to fall owing to loss of blood the suprarenals discharge adrenaline into the circulation, and this is said to accelerate the coagulation rate of the blood by an action on the liver.‡ If the liver is excluded from circulation the injection of adrenaline is without influence on the coagulability of the blood, and it also has no effect on it *in vitro*.

WHY DOES BLOOD NOT CLOT IN THE VESSELS ? No theory of coagulation can be satisfactory which does not account for this. One factor, at any rate, in the prevention of intravascular clotting must be the nature of the surfaces with which the blood comes in contact. Blood can for a time be prevented from clotting if it be kept from contact with any foreign substance which is wetted by it, as, for instance, when it is received into paraffined vessels. On the other hand, free contact with such substances, as occurs when the blood is defibrinated, hastens coagulation. One must therefore conclude that mere contact with a foreign body has a direct destructive action on the blood platelets. In birds, the platelets are more stable than in mammals, so that coagulation is held in abeyance if contamination with thrombokinase from the juices of damaged tissues is avoided. But birds' blood can be made to clot without the addition of tissue juice if, by violent whipping, filtration through a clay cell, or addition of water or chloroform, we destroy the formed elements, so leading to the liberation of their contained thrombokinase.

The modern tendency has, indeed, been to attribute the fluidity of blood in the vessels to the operation of an anti-coagulation factor, such as the presence of heparin, in the blood.

* BEST, COWAN and MACLEAN. *J. Physiol.*, 1938, **92**, 20.

† FULLERTON, *Lancet*, 1940 (ii), 195.

‡ GRAY and LUNT. *Amer. J. Physiol.*, 1914, **34**, 232; CANNON and MENDENHALL, *ibid.*, p. 243.

HEPARIN seems to prevent the conversion of prothrombin into thrombin, but Mellanby * showed it also acted as an anti-thrombin. Small amounts of thrombokinase liberated in, or injected artificially into, the circulation are neutralised by the liberation into the blood stream of equivalent amounts of heparin. There is good evidence that this heparin is formed in the Ehrlich's mast cells, which are found abundantly along the course of many blood vessels. These cells contain granules which stain purple with toluidine blue, a dye that precipitates heparin, and the intima of blood vessels yield, on extraction, quantities of heparin which are proportional to the abundance of the Ehrlich cells. Under certain conditions the heparin is rapidly poured out into the blood stream, *e.g.*, when peptone or small amounts of thrombin or thrombokinase are injected intravenously. In these circumstances not only does no coagulation take place, but the blood obtained after the injection will not clot.

INTRAVASCULAR CLOTTING. Intravascular clotting can be produced by the intravenous injection of small amounts of thrombin : thus 1 mg. of the solid precipitated from the thrombin solution made by Mellanby's method, when quickly injected into the cat's circulation, causes extensive clotting of blood in the vessels and immediate death. Intravascular clotting is still more easily caused by the injection of thrombokinase, *e.g.*, of normal saline extracts of tissues rich in cells, such as the thymus, lymph glands or testis.†

A curious result follows from injecting small amounts of these tissue extracts, or larger amounts very slowly. A minute quantity produces, not intravascular clotting, but a *delay* in the coagulation time. Repeated injections of small doses may absolutely annul the coagulability of the blood. The same double effect may be observed even with a larger dose. In rabbits, and in dogs after a full meal, the intravascular coagulation which occurs is complete, extending through the whole vascular system. If the injection be made into a fasting dog the thrombosis produced is limited to the portal vein. There is a sudden fall of blood pressure, from which the animal gradually recovers If a vessel be opened during the period of low pressure, the blood which flows out is uncoagulable, and if the animal be killed at this time a clot will be found filling up the whole portal vein. Wooldridge described these two effects of injection of tissue extracts, namely the coagulation and the loss of coagulability, as the *positive* and *negative phases* respectively. At least two different explanations have been given for the occurrence of the negative phase. Since it has not been observed *in vitro* it must be due to a reaction on the part of the living cells and probably to a secretion of heparin into the blood from the mast cells of the blood vessel walls, particularly in such organs as liver or lung. Another explanation has been suggested by Mellanby,‡ namely, that when small or slow injections of thrombokinase are given, intravascular coagulation actually does occur ; the fibrin formed is, however, taken up by tissue cells or slowly deposited on the walls of the blood vessels, so that these are not obliterated ; the blood then is really defibrinated blood, and so, of course, will not coagulate. Further evidence in support of this opinion has been given by the observation that slow injections of thrombin give exactly the same results § ; here also the slow injection causes slow continuous coagulation, but the fibrin so formed is safely deposited in places where it can do no harm. There is no reason why both these explanations may not be true in part.

* J. MELLANBY. *Proc. Roy. Soc.*, 1934, **116B**, 1.
† WOOLDRIDGE. "Chemistry of the Blood," 1893. (Kegan Paul, London.)
‡ MELLANBY. *J. Physiol.*, 1909, **38**, 474.
§ MELLANBY. *Proc. Roy. Soc.*, 1933, **113B**, 93

Fate of the Thrombin. The substances which interact for the production of thrombin in shed blood, and thrombin itself, are not entirely used up in the process of clotting. Blood serum contains traces of thrombokinase (which can be precipitated by the addition of dilute acetic acid) and prothrombin, as well as a fairly strong solution of thrombin. Thrombin, however, rapidly disappears from serum, which in two or three days may be almost free from it. It has been suggested that the thrombin undergoes a modification into an inactive form, called *metathrombin*. This is unaltered by calcium salts or by thrombokinase, but can be reconverted into thrombin by traces of acids or alkalies. According to Rettger the disappearance of thrombin from serum is due to its combination with some of the proteins of the serum. This combination, like that of thrombin with fibrinogen to form fibrin, is unstable and can be broken up by the action of alkalies, acids or by putrefaction.

We are now in a position to explain the behaviour of the various kinds of plasma.

Cooled Plasma contains the thrombokinase in the blood platelets. These can be separated by centrifuging at a low temperature or by filtration. The remaining plasma contains only prothrombin, calcium salts and fibrinogen, and can be made to clot by the addition of tissue extracts or of thrombin, but not by warming.

In Sodium Sulphate Plasma the interaction of the fibrin factors is merely impeded by the excess of salt. All are still present, and it is therefore sufficient merely to dilute the plasma in order to produce clotting.

Magnesium Sulphate Plasma if centrifuged while still warm will clot on simple dilution. If the blood be left for twenty-four hours before centrifuging, the plasma will not clot on dilution nor on addition of tissue extracts. It contains fibrinogen only, and is therefore an excellent reagent for the presence of thrombin. Magnesium sulphate not only hinders the interaction of the fibrin factors, but actually slowly precipitates the prothrombin and thrombin.

Sodium Fluoride Plasma might be expected to act like oxalate plasma but fluoride has the additional property of causing fixation of the formed elements of the blood, including the blood platelets. If it be thoroughly centrifuged so that the plasma is obtained free from these constituents, it will no longer clot with calcium salts nor even with calcium salts *plus* tissue extracts, but will clot readily on addition of thrombin. Although it still contains a certain amount of prothrombin, this is entangled and carried down in the precipitate of calcium fluoride which is produced by the addition of calcium salts, so that the thrombokinase has nothing on which to exercise its effect. Sodium fluoride plasma is therefore useful as a test for the presence of thrombin. If water be added to the sodium fluoride blood, so as to destroy some of the formed elements and liberate their constituents into the plasma, it is possible to produce clotting by the simple addition of calcium salts.

Hirudin Plasma. The action of hirudin is that of an anti-thrombin. It apparently combines with and neutralises thrombin. Hirudin plasma can therefore be made to clot by the addition of thrombin in sufficiently large quantities to combine with all the hirudin present and leave an excess over in the fluid.

Heparin Plasma remains fluid because the conversion of prothrombin to thrombin is inhibited. It clots on dilution, or on treatment with carbon dioxide, because kinase is thus set free, which then neutralises the heparin. It also clots on addition of toluidine blue or protamine; these basic substances precipitate the strongly acidic heparin.

Peptone Plasma. If blood be received into peptone blood, obtained by the injection of large doses of peptone into the veins of another animal, the mixture does not clot. This is because the peptone blood contains an excess of heparin, which can in fact be extracted from it.* It has been shown by Delezenne and others that the seat of formation of the anticoagulin is in the liver. In anaphylactic shock also the blood is incoagulable because of the presence in it of an excess of heparin.

The Transudations. The earlier work on coagulation was largely carried out on the fluids obtained from the pericardial or pleural cavities, or on hydrocele fluid from the *tunica vaginalis*. These as a rule can be kept

* Wilander. *Skand. Arch. Physiol.*, 1938, **81**, Suppl. XV.

indefinitely without clotting, but will clot readily on addition of a few drops of blood, or of thrombin in any form. They will not clot on the addition of tissue extracts. Their behaviour is readily explained by the assumption that they contain fibrinogen, but are free from thrombokinase and prothrombin. In order to produce coagulation it is therefore necessary to add both these factors, as happens when we add blood, or else to add thrombin.

THEORIES OF COAGULATION. The incompleteness of our knowledge of what really happens in clotting is reflected in the great variety of theories which have been advanced to explain it. Any theory should have regard to the following facts: (1) Plasma contains all the substances necessary for clotting. (2) Blood does not clot in normal blood vessels. (3) Coagulation is closely related to the properties of the colloids concerned in it. (4) Essential factors in clotting are primarily fibrinogen and thrombin; secondary factors which govern the formation of thrombin are prothrombin, calcium salts and thrombokinase. (5) In hæmophilia the blood is only slowly coagulable or even incoagulable. Thus far, though with different nomenclatures, most workers are now agreed.

Morawitz called the thromboplastic factor thrombokinase, and considered that this substance, liberated by platelets or damaged tissues, in presence of calcium salts, converted a precursor, prothrombin in the plasma, into thrombin.

According to Nolf the essential factors are three proteins: fibrinogen, thrombogen and thrombozym. The two former are produced in the liver, while the thrombozym is formed from the leucocytes. The clotting depends on a mutual interaction and precipitation of colloids with, as a result, either fibrin or thrombin. Thrombin differs from fibrin merely in containing less fibrinogen. For this reaction to take place, the presence of calcium is necessary as well as certain thromboplastic substances which act as centres of precipitation. Thrombin, according to him, is merely an unsaturated compound which is capable of taking up or uniting with more fibrinogen to form fibrin. He compares these actions to the actions of digestive enzymes on proteins; fibrinogen is first precipitated as fibrin by union with thrombozym and thrombogen. This fibrin is then hydrolysed and dissolved by the further action of the thrombozym, which he regards as essentially proteolytic in character. That the blood does not coagulate within the vessels he explains by assuming that the cells of the blood and tissues are covered normally with an ultra-microscopic layer of fibrin which has no thromboplastic effect upon the plasma.

Howell's theory assumes that the thromboplastic material (cephalin) liberated by platelets or by other damaged tissue cells neutralises heparin, which normally maintains the fluidity of the blood *in vivo*, so that calcium salts can then convert prothrombin to thrombin.

Mills * and Pickering regard the various plasma colloids as forming a complex by physico-chemical union, instead of being mere mixtures of the various products such as can be artificially separated from the plasma. Under the conditions which obtain in the vessels the equilibrium of this complex is stable, but when the blood is shed various factors disturb this delicate balance.

It seems certain that complex physico-chemical processes connected with electric charges on colloids or high-molecular substances play an important part, since many anti-coagulating substances are strongly acidic or basic substances of large molecular weight. For instance, the lignine sulphonic acids (Liquid Roche), and Germanin and heparin are acidic; Janus green is basic.

HÆMORRHAGIC STATES. In some pathological states the bleeding from a wound is abnormally prolonged. This may be due to one or more of many causes. Sometimes the condition is hereditary and is called *hæmophilia*.

In this condition the blood clots, if at all, with extreme slowness; hence subjects suffering from the complaint may die of hæmorrhage as a result of trivial injuries. Yet their blood undoubtedly contains prothrombin and fibrinogen, so that if thrombokinase solution is added it clots normally. The most probable explanation is that the platelets of hæmophilic blood are less readily made to yield sufficient thrombokinase than those of normal blood, either because of their small number or their relative stability. Local application of certain snake venoms to the wound accelerates clotting.

* MILLS. *J. Physiol.*, 1936, **62**, ix.

The disorder is of further interest because it is hereditary and is transmitted through the females, who are not affected by it, to the male descendants through many generations.

In other cases there is a lack of prothrombin, as in jaundice and in certain nutritional disorders, due to lack of vitamin K; or there may be lack of fibrinogen, as in gross liver damage or in the hereditary pseudohæmophilia. Very rarely, if ever, is excessive bleeding due to calcium deficit, because for this to occur the $Ca^{\cdot\cdot}$ would have to be reduced to a level incompatible with life. A primary lack of some clotting factor is, however, not the only cause of excessive bleeding. For instance, in anaphylactic shock the blood is incoagulable because of the presence of an excess of heparin.

Often the hæmorrhagic state is associated with the occurrence of purple or livid spots on the skin--a symptom called purpura—which is due to fragility of the capillary walls. This may be associated with reduced blood coagulability, as in the purpura due to lack of vitamin K, but often the coagulation time is normal and there is no detectable lack of any coagulation factor in the blood. The most outstanding of such examples is scurvy, due to lack of vitamin C; another may be due to lack of another vitamin, called vitamin P (flavone) so raising the permeability of the blood capillaries. Again, some states of purpura (idiopathic thrombocytopenic purpura) are distinguished by a reduction in the number of blood platelets. Whether this is due to sluggish production or accelerated destruction of platelets is unknown, and the condition is often relieved by removal of the spleen. Platelet deficiency is also present in the purpuras of aplastic anæmia, and lymphatic leukæmia. Some believe that the primary fault is in the capillary endothelium, which is excessively vulnerable; hence, many platelets are used up to repair the breaches, with consequent lowering of the circulating platelets.

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THE CIRCULATION

CHAPTER XXX

GENERAL FEATURES OF THE CIRCULATION

IN order that the nutrition of the tissues may be properly carried out, and that they may be able to free themselves of their waste products, the blood which flows through them must be continually renewed. For this purpose

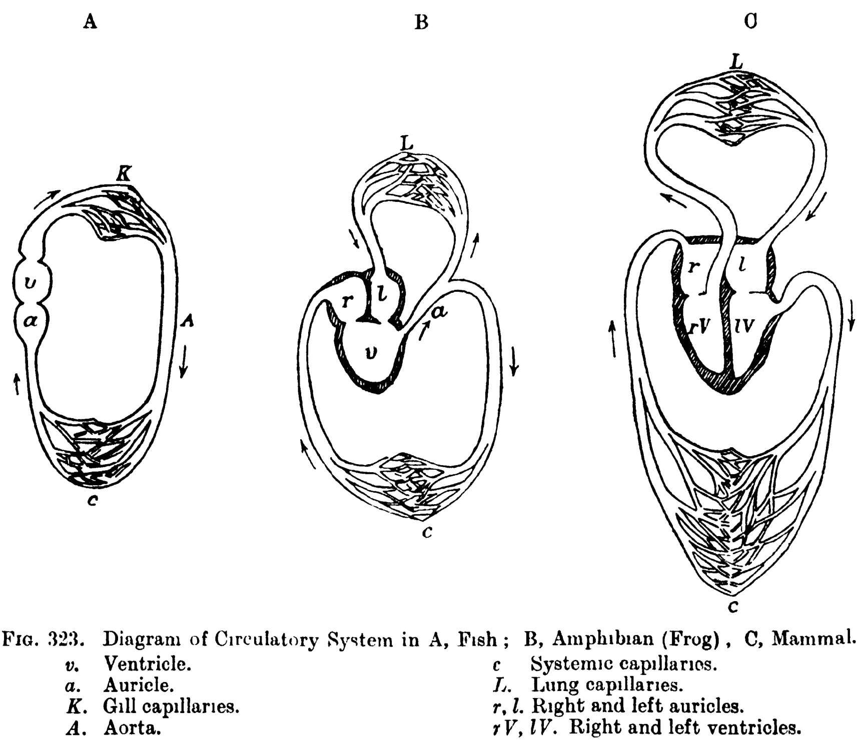

FIG. 323. Diagram of Circulatory System in A, Fish; B, Amphibian (Frog), C, Mammal.

v. Ventricle.	c Systemic capillaries.
a. Auricle.	L. Lung capillaries.
K. Gill capillaries.	r, l. Right and left auricles.
A. Aorta.	rV, lV. Right and left ventricles.

every part of the body is supplied with tubes—blood vessels—of varying size and structure.

In the tissues the blood is passing continuously through a close meshwork of capillaries, minute vessels with walls consisting of a single layer of delicate endothelial cells, which permit of a free interchange of material by diffusion between the blood within and the tissue fluid outside the vessel. The movement of the blood is maintained by the heart, which acts as a pump, transferring the blood it receives at the low pressure of the veins, to the arteries, at a high pressure, the blood being taken from the heart to the tissues by the arteries, and carried back from the tissues to the heart by the veins.

In all the vertebrates the vascular system is closed, *i.e.* communicates at no point with the tissue spaces or cœlomic cavity. It is found in its simplest form in fishes (Fig. 323, A), where the heart consists of one auricle and one ventricle. The blood is

received from the great veins into the auricle. The walls both of auricle and ventricle contract rhythmically. By the contraction of the auricle the blood is forced into the ventricle and this, when it contracts, sends the blood on into the bulbus arteriosus. From the bulbus the blood passes through the branchial arteries into the gills, where it takes up oxygen from the surrounding water, and then flows on into the aorta, by which it is distributed to the various organs of the body. From the capillaries of these organs the blood is collected by the veins and is carried once more back to the auricle. The fish heart is thus entirely on the venous side of the vascular system.

In amphibia, such as the frog (Fig. 323, B), the heart consists of two auricles and one ventricle. The right auricle receives venous blood from the body by means of the venæ cavæ and forces it by its contraction into the ventricle. From the ventricle the blood passes into the aorta, whence it is carried partly by the pulmonary artery to the lungs, partly by arteries to the different organs of the body. The blood, which has passed through the lungs and been oxygenated, flows through the pulmonary veins to the left auricle, whence it passes into the ventricle and mixes with the venous blood which is arriving from the right auricle. The pulmonary circulation is thus merely a branch of the

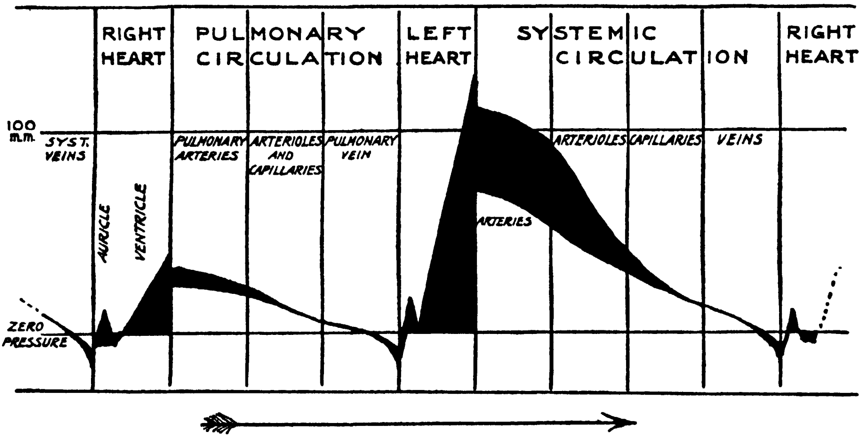

FIG. 324. Diagram to illustrate the pressures in different parts of the vascular circuit. The circulation proceeds in the direction of the arrow. Lines of "zero" (= atmospheric) pressure and of 100 mm. Hg pressure are shown. The pressures are shown as a graph of varying breadth; the breadth at any point indicates the approximate magnitude of the pressure fluctuations caused by the heart beats. These fluctuations are at a maximum in the ventricles and at a minimum in the smallest capillaries and veins, in which the pressure is almost constant. (The fluctuation in the pulmonary arteries is probably much greater than is here shown.)

general or systemic circulation. The bulbus aortæ in the frog is divided into two parts by means of a spiral valve, by which a partial separation of the blood coming from the right and left auricles is effected, and the venous blood from the right auricle directed especially into the pulmonary artery.

In birds and mammals the heart has become entirely divided into two halves, right and left, which have no communication with one another except by way of the blood vessels and capillaries. The right auricle receives the venous blood from all parts of the body and sends it on to the right ventricle, whence it is forced into the lungs along the pulmonary artery. In the lungs it takes up oxygen and so becomes "arterial" and is returned by the pulmonary veins to the left auricle and on to the left ventricle. The rhythmic contractions of the left ventricle then force the blood into the aorta, whence by the branching arteries it is carried to all parts of the body (Fig. 323, C).

The whole vascular system is distensible and elastic, so that its capacity will increase with the pressure of the blood contained in it. Since the driving force is furnished by the heart, the pressure which causes the flow of blood through the system must decline as we pass from the arterial to the venous side (Fig. 324). In the arteries the pressure is high, *e.g.* up to about

120 mm. Hg in the large arteries of man ; it is somewhat lower in the smaller arteries. The chief function of the large arteries is to serve as elastic conduits, whereas the small arteries and arterioles leading from the arteries to the capillaries have in addition the function of regulating the amount of blood flowing through the capillary area of the organs which they supply. In the capillaries the pressure is much lower, up to, say, 30 to 50 mm. Hg. The veins have the function of conducting blood at a low pressure (not usually greater than 10 mm. Hg) from capillaries to heart and of storing up any excess of blood which is not immediately taken up by the heart.

Corresponding to this difference in function we find variations in the structure of the blood vessels according to their situation in the circuit. The arteries are thick-walled, and contain an abundance of muscular and elastic elements in their walls. The typical medium-sized artery is described as consisting of three coats (Fig. 325) : an *intima* lined by a continuous layer of flattened endothelial cells, which rest on a well-marked lamina of yellow elastic tissue ; a *media* composed of unstriated muscular fibres arranged mainly circularly ; and an external coat or *adventitia* of fibrous tissue, with a number of longitudinal elastic fibres. Near the heart, in the great vessels such as the aorta and its larger branches, there is a preponderance of elastic tissue as compared to the muscular ; and we find in the media alternate layers of muscle fibres and fenestrated elastic mem-

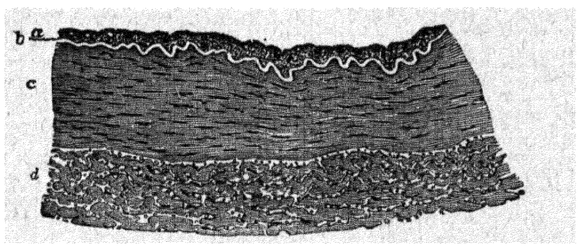

FIG. 325. Transverse Section of Part of the Wall of the Posterior Tibial Artery (× 75). (SCHAFER.)

a. Endothelial and sub-endothelial layers of intima.
b. Lamina of elastic tissue.
c. Media consisting of muscle fibres
d. Adventitia.

branes. Arteries are of two types, the " elastic " and the " muscular." The aortic arch and its main branches are of the " elastic " type ; they constitute a large elastic reservoir which lightens the work of the heart and converts the intermittent output into a continuous flow. The smaller arteries, such as radial and lingual, are of " muscular " type ; they serve to regulate the supply of blood to the various organs as their needs require it. In the smallest arteries, the arterioles, the elastic element entirely disappears, so that the wall consists of muscle fibres, chiefly circular, lined by the endothelium. In these vessels a contraction of their walls may result in an entire obliteration of the lumen, so shutting off altogether the supply of blood to the capillaries beyond. The capillaries, though formed merely of a single layer of endothelial cells, also possess the power of contracting and relaxing, and so admitting of a larger or smaller flow of blood to the tissues according to their local needs. In the veins the same three coats can be distinguished as in the typical artery, but the wall of the vessel is much thinner in proportion to the lumen. In the vein, moreover, there is a preponderance of the fibrous tissue elements, the muscular and elastic tissue being but little marked. On this account the vein collapses unless it is distended by some internal pressure.

The histological difference between veins and arteries is of importance for the understanding of the distribution of pressures in the vascular system, since the distensibility of these vessels is conditioned by their structure. In Fig. 326 is represented the distensibility, *i.e.* the increase in capacity, of a vein under gradually increasing internal pressure. It will be seen that the capacity, which is negligible at zero pressure, becomes considerable on raising

the pressure to 1 mm. Hg. A further rise of pressure to 10 mm. Hg causes a very large increase in volume, but from this point the increments of volume with rising pressure rapidly diminish. On the other hand, the artery, which has a certain capacity at zero pressure, increases its capacity much more slowly with rise of pressure, and the increment of capacity becomes gradually less as the internal pressure rises. In Fig. 327 is shown the distensibility of an artery to sudden alterations of pressure such as occur with each heart beat. It indicates that the arterial wall becomes more rigid with increasing distension. In the thoracic aorta of young subjects the curve is S-shaped, the most rapid distension taking place at about 100 mm. Hg. pressure.* The aorta is thus well suited to expand at each systole.

As the arteries branch, although each branch is smaller than the parent vessel, the total area of the two branches into which the vessel divides is greater. Thus there is a continual increase in the cross area of the bed of the blood stream as we pass from the heart towards the periphery. This increase is especially marked at the junction between the capillaries and the arterioles on one side and the venules on the other, so that the total area

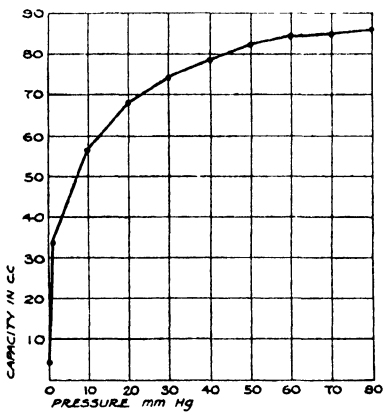

FIG 326 Vein.

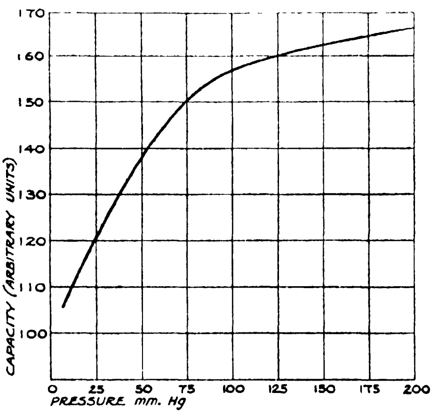

FIG 327. Artery

of the bed in the region of the capillaries can be taken as about 800 times that of the area of the aorta where the blood leaves the heart.

This figure would apply only to normal conditions, under which but a portion of the capillaries are open and pervious to the blood current. By the opening up of previously closed capillaries, the capacity of the capillaries in active muscle may be increased to many times that of these vessels in a resting muscle. In fact, a general and simultaneous dilatation of all the capillaries of the body may increase the capacity of the stream bed to such an extent that there is an insufficient return of the blood to the heart, and a condition of ' surgical shock ' is brought about.

Arterio-venous Anastomoses. Although the bulk of the circulating blood has to pass through capillaries in order to travel from the arterial to the venous system, this is not invariably the case, for in certain parts of the body there are direct channels leading from arteries to veins. These are called arterio-venous anastomoses. In them the arterial twig, after a more or less tortuous course, opens directly into a relatively wide, thin-walled vein. The structures are often collected together in groups in certain situations, or may be scattered singly. They occur especially in erectile tissue, such as the penis, in the skin of the balls of the fingers, in the nail bed, and to a remarkable

* HALLOCK and BENSON. *J. Clin. Investig*, 1937, **16**, 595, WIGGERS. *Amer. J Physiol.* 1938, **123**, 644.

degree in the rabbit's ear skin. The openings of these anastomoses are capable of being closed by the contraction of a thick muscular wall; when this is relaxed the arterial blood is short-circuited directly into the vein.

DISCOVERY OF THE CIRCULATION. The fact that blood can pass from the right to the left ventricles by way of the lungs—the pulmonary circulation—was first advanced by Ibn Nafīs, an Arabian physician, in the thirteenth century,* and again independently by Michael Servetus and four others in the sixteenth century. Servetus, however, only believed that part of the blood passed through the lungs.†

The discovery that the blood travels round in a circuit, returning again and again to the same point, was made by William Harvey, physician to Charles I., and was given to the world in a book of seventy-two pages, printed at Frankfurt in 1628.‡

This, the greatest discovery in the whole of medical science, of which indeed it laid the foundation, was the result of prolonged experiment and patient observation, but in his masterly though modest treatise Harvey shows that the circulation is a logical necessity to which we must be led by the contemplation of certain elementary facts.

(1) The arrangement of the valves in the heart and veins is such as to allow of a movement in one direction only.

(2) 'If the heart is grasped in the hand, it may be felt to become harder during its action.' When this happens, the ventricles become smaller and the arteries expand; if an artery is severed, blood spurts from its central end at each heart contraction.

(3) Since, at each contraction of the heart blood is forced into the arteries and cannot return, it follows that, even if only a small quantity were so expelled, there would not be enough blood in the heart, or even in the whole body, for the process to continue, nor could all this blood remain in the arteries. The heart must, therefore, receive blood continuously from the veins and pass it out continuously into the arteries. If a vein is compressed or tied by a ligature, it swells up on the side furthest from the heart, and collapses on the side nearest the heart. With an artery it is the reverse. Hence the blood must be passing continuously from the arteries, through the tissues into the veins. Similarly, the blood in the right ventricle must pass by way of the pulmonary artery and lungs to the pulmonary veins and so reach the left side of the heart.

Harvey could not be certain how the blood passed through the tissues from arteries to veins, but with the use of the microscope, the capillaries were discovered by Malpighi in 1661,§ and the missing link in the argument was thus provided.

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* SAMI HADDAD and AMIN KHAIRALLAH (1936) *Annals of Surgery*, 104, 1; MAX MEYERHOF (1936). *Isis*, **23**, 100. (1933) *Bull de l'Instit d'Égypte*, **16**, 33.

† IZQUIERDO *Bull Inst. Hist Med*, 1937, **5**, 914

‡ HARVEY. "Exercitatio Anatomica de motu cordis et sanguinis in animalibus," Frankfurt, 1628. Many editions of English translations may be had.

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§ MALPIGHI. "De Pulmonibus," 1661.

CHAPTER XXXI

PHYSIOLOGY OF THE HEART

CARDIAC MUSCLE

In the ordinary span of a man's life his heart performs about 2,600 million beats, pumping at least some 155 million litres (say about 150,000 tons) of blood from each ventricle : at the lowest estimate the work done amounts to 241 million kg.-m., enough to raise a weight of 10 tons to a height of ten miles. In all this performance scarcely a beat is misplaced, and there is no rest longer than about three-quarters of a second throughout. It must

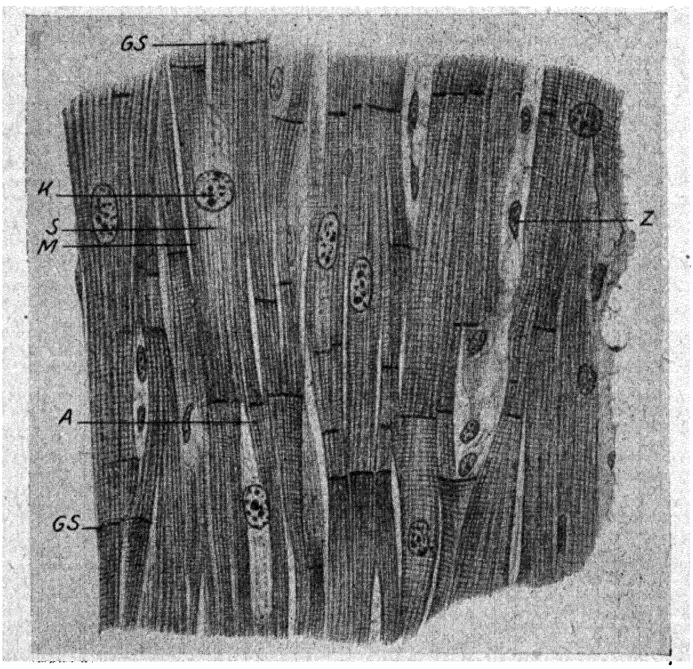

Fig. 328. Cardiac muscle, showing the cross- and longitudinally-striated cells, with centrally placed nuclei, K, connected with adjacent cells at either end, GS, and also laterally by means of the branches, A. (Schaffer, *Vorlesungen über Histologie u. Histogenese.*)

be admitted that this is a remarkable performance for an organ weighing only about 300 grm., even when spread over seventy years.

If the heart be cut out of the body of a cold-blooded animal it will continue to beat with the normal sequence of its different chambers for hours, or even days, provided that it be kept cool and moist. In the case of a warm-blooded animal, the heart is similarly capable under proper conditions of continuing its rhythmic contractions for many hours after excision. The fact that the

heart will continue to beat after removal from the body, shows that the causation of the beat is to be sought in the walls of the heart itself. The heart wall consists of cardiac muscular tissue; it presents longitudinal and transverse striations and is capable of contracting both 'spontaneously' and in response to direct stimulation. The histological unit is a muscle cell, roughly quadrilateral when viewed from the surface. Each cell has a nucleus near its centre, and is joined to adjacent cells to form fibres. No sarcolemma is present, and the fibres lying side by side are connected at frequent intervals by short bridges (Fig. 328). The contractile units are thus not separate, as in voluntary muscle, but are in protoplasmic and functional continuity, *i.e.* they form a *syncytium.*

ANATOMY OF THE HEART IN THE FROG AND TORTOISE. The hearts of the frog and of the tortoise have figured largely in the researches on the causation of the heart beat.

The frog's heart (Fig. 329 consists of the sinus venosus, which receives the anterior and posterior venæ cavæ, two auricles, one ventricle, and the bulbus arteriosus which opens into the two aortæ. The venous blood from the body flows into the sinus venosus by the three venæ cavæ, and thence into the right auricle, while the left auricle receives the blood from the lungs. The ventricle thus receives mixed arterial and venous

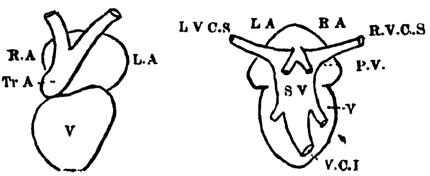

FIG. 329. Diagram of Frog's Heart. (After CYON)
V, ventricle; R.A., L.A., right and left auricles (atria); S.V., sinus venosus; P.V., pulmonary veins; L.V C.S. and R.V.C.S, left and right anterior vena cava; V.C.I., vena cava posterior; TR.A., bulbus arteriosus.

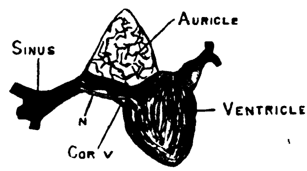

FIG. 330. Tortoise's Heart (after GASKELL) as it appears when Suspended for Registering the Auricular and Ventricular Contractions. N, nerve trunk with fibres connecting Remak's and Bidder's ganglia; COR. V., coronary vein.

blood, the arterial blood being directed by the spiral valve of the bulbus aortæ so as to flow chiefly towards the head.

The muscular fibres of the frog's heart are less highly developed than those of the mammalian heart. They are spindle-shaped, and only dimly cross-striated. The cross-striation becomes more distinctly marked as we proceed from sinus to ventricle, the sinus muscle fibre representing the most primitive condition. There is complete muscular continuity between all the cavities of the heart. The circular ring of muscle at the junction of sinus with auricles and of auricles with ventricle presents only slight traces of cross-striation.

The heart is well supplied with nerve fibres and ganglion cells. The two vagi enter the sinus venosus and branch just under the pericardium. Here they become connected with a collection of nerve cells, known as Remak's ganglion. From the sinus the two vagi, now called septal nerves, pass down in the interauricular septum, one in front and the other behind. Near the auriculo-ventricular groove they enter two collections of ganglion cells, called Bidder's ganglia. From these ganglia non-medullated fibres are distributed to surrounding parts of the auricle and to the whole of the ventricle. In the upper third of the ventricle occur scattered ganglion cells attached to the nerve fibres. These are quite absent in the lower half or two-thirds.

In the tortoise (Fig. 330) the two auricles are bound together by a flat band of tissue, which serves also to connect the sinus with the ventricle. The septum between the auricles arises from the central line of this junction wall. The two vagus nerves pass into a large accumulation of ganglion cells in the sinus, and thence along the basal wall to the auriculo-ventricular groove, lying just under the pericardium. In the groove they pass into a collection of ganglion cells, whence fibres are given off to both auricles and ventricle. As they leave the sinus, a branch of the right nerve runs along the coronary vein, which conveys blood from the ventricular wall to the sinus.

THE CONTRACTION OF THE COLD-BLOODED HEART

THE STANNIUS LIGATURES. The frog's heart beats regularly, the contraction starting in the sinus, then travelling to auricles, ventricle and bulbus. If, however, the auricles be functionally separated from the sinus by a ligature round this junction (first Stannius ligature), the auricles and ventricle stop in an uncontracted condition (diastole), while the sinus goes on beating regularly. After the lapse of a period varying from five minutes to half an hour, the detached part of the heart begins to beat, at first slowly and then more rapidly, but never attaining the rate of the sinus. The auricles beat first and then the ventricle. If now the ventricle be separated from the auricles by a ligature at the auriculo-ventricular groove (second Stannius ligature), the auricles continue to beat, while the ventricle, after a few beats due to the excitation of the ligature, stops beating, though after a considerable time it may begin again to contract very slowly. But the lower two-thirds of the ventricle, if separated from the rest of the heart, never beats again, under normal circumstances. To single stimuli it responds with a single beat, not with a series of beats as the whole heart does. It can be made to beat rhythmically, however, by raising the pressure within its cavity.

There is thus a descending scale of automatic power in the different parts of the frog's heart—from the sinus, where it is highest, to the lower parts of the ventricle, where it is very slight. The normal sequence of events—*i.e.* the subordination of the ventricle to auricles, and auricles to sinus, so that the beat always follows in the order, sinus, auricles, ventricle, bulbus—can be ascribed to the difference between the natural rhythms of these different cavities. It is possible to record the contractions of each of these parts of the heart separately, after having divided them, either functionally by crushing the intervening tissue, or by actual section. Under such conditions it is found that there is a descending scale of rhythm from sinus to bulbus, the contractions of the sinus being most frequent, those of the ventricle and bulbus the least frequent. Thus it is impossible for the ventricle to function at its own rhythm, since before it is ready to beat again spontaneously after performing one contraction, it receives an impulse from the auricles which induces a beat. That the normal sequence of contractions is dependent on the domination by the sinus, is shown by the fact that, by exciting the ventricle by means of induction shocks repeated at a rhythm slightly quicker than that of the sinus, it is possible to induce a reverse rhythm, the order of the beat being now ventricle, auricles, sinus venosus. It is clear, therefore, that the portion of the heart which beats most rapidly, dominates the rest, and is therefore called the *pace-maker*.

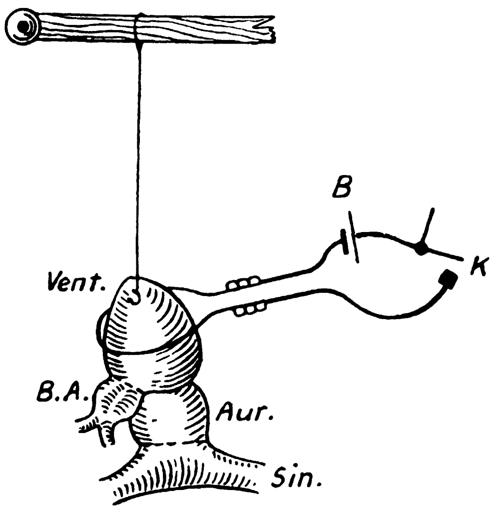

FIG. 331

That the sinus is the pace-maker in the frog's heart may be shown by a simple experiment. The ventricle is connected with a recording lever, and a platinum loop is put

round the heart, either round the sinus or round the ventricle (Fig. 331). When a current is passed through the wire loop, the corresponding part of the heart is warmed. When the ventricle alone is warmed the rhythm is unaltered. On lowering the loop so as to warm the sinus, the rhythm of the whole heart is quickened.

The different rhythmic power of these parts of the heart is apparently connected with the histological characters of the muscle fibres at each part. The lowly differentiated sinus cell has well-marked rhythmic power and a quick rhythm of beat, but is not able to exert much force in its contraction. The more highly differentiated ventricle cell has only a slight rhythmic power, but beats forcibly.

NEUROGENIC THEORY OF HEART BEAT. It was formerly thought that the ganglia (Remak's and Bidder's ganglia) were responsible for the initiation and co-ordination of the beats. This theory has been abandoned since it was shown by Gaskell and by Engelmann that—

(*a*) These ganglia may be excised in the frog and tortoise without altering the contractions or normal sequence of beat.
(*b*) Remak's ganglia are merely distributing ganglia for the vagus nerves to the heart. They may be paralysed by nicotine without disturbing the beat.
(*c*) A small portion of cardiac muscle, entirely free from ganglion cells, may be seen to contract rhythmically.
(*d*) The heart in the developing chick begins to contract at a date prior to the growth into it of nerve cells.

Finally it has been shown that isolated cardiac muscle cells, in tissue culture outside the body, show rhythmic contractions.

THE PROPAGATION OF THE WAVE OF CONTRACTION. The normal contraction is started in the sinus venosus, propagated to the auricles, thence to the ventricle, and finally to the bulbus aortæ. Between the contractions of each of these cavities there is a slight pause, whereas the contraction spreads so rapidly over each cavity that all parts, say of the auricles or ventricle, appear to contract simultaneously. Cardiac muscle forms a syncytium, and the excitory wave is propagated through the heart from one muscle cell to another, and not by means of nerve fibres.

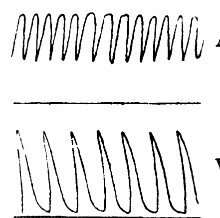

FIG. 332. Contraction of Auricles and Ventricle of Tortoise Heart The auriculo-ventricular groove has been clamped so as to produce a partial block allowing only every second contraction to pass. (GASKELL.)

That the propagation cannot be due to any nerve trunks running from sinus to ventricle is shown by various experiments of Engelmann and Gaskell.* Thus, if the auricle is slit up by a series of interdigitating cuts, the contraction wave starting from the sinus travels along the auricular muscle around the end of each section and finally, on arrival at the ventricle, causes a contraction of this cavity. In the heart of the tortoise, the nerve trunks run in the band of tissue joining the sinus to the ventricle; this band, with all its contained nerves, can be excised without interfering in any way with the normal sequence of contractions.

The pause observed between the contractions of auricles and ventricle has been shown by Gaskell to be due to a partial "*block*" in the excitatory wave which occurs in its propagation through the muscular tissue in the auriculo-ventricular junction. A similar retardation of the wave can be produced at any point, in either auricles or ventricle, by compression by a clamp, or by cutting away the conducting muscular tissue to a sufficiently small amount. If the normal auriculo-ventricular block be increased, as for instance by compression of the auriculo-ventricular groove by means of a clamp, it may be so increased that only one out of every two or more auricular contractions passes across to the ventricle (Fig. 332), and further

* GASKELL. *J. Physiol.*, 1883, **4**, 43.

increase in the resistance to the propagation of the wave may lead to the block becoming complete.

The wave of excitation is believed to pass from cell to cell of the cardiac muscle by means of the intercellular bridges with which these interconnect.

By the methylene blue method it is possible to demonstrate a close network of non-medullated fibres surrounding all the muscle cells of the heart. It is obvious that the experiment just quoted would not exclude the possibility of propagation occurring through such a nerve network, but the properties of the network would have to differ from those of any of the nerve tissues with which we are acquainted; for example, the velocity with which the excitation is forwarded is much slower than that in nerve.

The progress of the excitatory wave is well seen if a record be taken of the electrical changes resulting in the frog's heart from a single stimulation. A series of electrical changes occurs, which can be interpreted as due to the passage of a wave of excitation from the base to the apex of the heart.

THE PROPERTIES OF CARDIAC MUSCLE

Since both the rhythm of the heart and the propagation of the contraction from one cavity to the next are functions of the cardiac muscle, a further consideration of the properties of this tissue is essential to enable us to decide to what extent the behaviour of the heart under varying conditions may be referred to the elementary properties of its muscular wall.

Rhythmicity. The cause of the spontaneous rhythm of the various portions of the heart, and of the higher rhythm of the sinus tissue, is not known. It is evident that some stimulating agent is present in the cardiac cells themselves, and attention has recently been given to the possibility that this stimulus is a chemical one, as in the 'heart hormone' of Haberlandt * or the 'active substance' of Demoor.† The higher rhythmic activity of the sinus is, according to these views, due to the more rapid production of the 'hormone' in that region. According to others it is the outcome of a more vigorous metabolism.

Response to Direct Excitation. The curve of contraction of cardiac muscle resembles that of voluntary muscle, except that it occupies a longer time; the contraction of the mammalian ventricular muscle lasts three-tenths of a second, of the frog's ventricle about half a second, and of the tortoise ventricle about two seconds. The contractile process originates at a stimulated point and travels thence to all other points.

ALL-OR-NONE CONTRACTION. If a ventricle strip be stimulated with a single induction shock, if it responds at all it will respond with a maximal contraction, no increase being obtainable, however the stimulus may be increased.‡ There is thus no proportionality in the heart between strength of stimulus and height of contraction. We have seen that the response to a minimal stimulus in a skeletal muscle is smaller than the response to a maximal stimulus, because in the former case only a small proportion of the muscle fibres is active, so that increasing the strength of the stimulus merely increases the number of fibres thrown into contraction, and a maximal contraction of skeletal muscle is one involving all the fibres. In the heart muscle all the muscle fibres are functionally continuous, so that a stimulus if it excites at all, must excite all the fibres, and every contraction must be

* HABERLANDT. *Klin. Woch.*, 1924, **3**, 1631; *Pflüger's Arch.*, 1929, **223**, 282. HABERLANDT. "Das Hormon der Herzbewegung," 1927.

† DEMOOR, *Arch. Internat. de Physiol.*, 1924, **23**, 121; DEMOOR and RYLANT, *Compt. rend. Soc. Belge. de biol.*, 1925, **2**, 814; *Arch. Internat. de Physiol.*, 1930, **32**, 80.

‡ BOWDITCH. Ber. Sächs Ges., 1871, **23**, 652.

analogous to the maximal contraction of a skeletal muscle. The 'all-or-none' law is therefore demonstrable without special methods in heart muscle.

SUMMATION OF STIMULI. If an isolated frog's ventricle which is not beating be stimulated with inadequate shocks, it may be found, on repeating these shocks at short intervals of time, that they culminate to cause a contraction of the ventricle. Stimuli which are subminimal may therefore summate.

STAIRCASE. An improving effect of previous stimulation on the condition of heart muscle may be observed on the contractions themselves. Thus in a 'Stannius preparation,' if the ventricle be excited with single induction shocks once in every ten seconds, the first four or five contractions form an ascending series, each contraction being rather higher than the preceding one. This is often spoken of as the 'staircase phenomenon' (Fig. 333). Each of the contractions represents a maximal contraction, but this maximum effort is at first not so great as it becomes after a few previous contractions. The phenomenon appears to be due to the accumulation of products of contraction which are beneficial.*

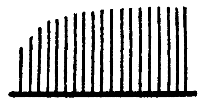

FIG. 333 Group of Contractions showing 'Staircase' character.

THE REFRACTORY PERIOD. When two stimuli are applied to any tissue at a sufficiently short interval of time, the second stimulus is without effect. In the case of skeletal muscle, the refractory period has passed away long before the muscle begins to respond, so that the stimulus applied after recovery from the refractory stage excites a second response, and wave-summation is produced; repeated stimuli give a tetanus.

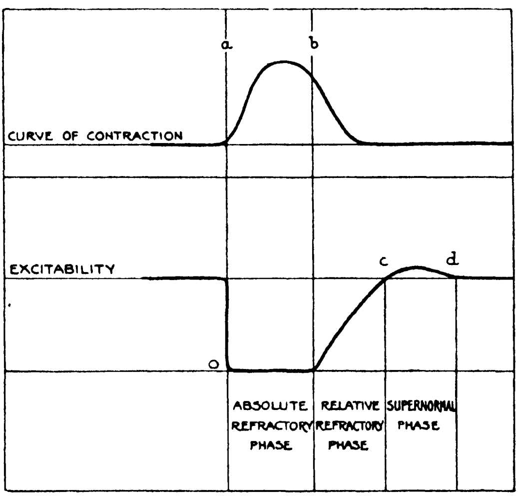

FIG. 334. Diagram showing changes in the excitability of heart muscle accompanying and following a single contraction.

In heart muscle the refractory period is extremely prolonged, *lasting as long as the contraction*, whether this occurs spontaneously or is evoked by a single stimulus. Hence, during the whole time that the muscle is contracted, any stimulus, however strong, applied to it remains without effect. Heart muscle therefore cannot be tetanised. As the contraction passes away the irritability once more rises and, as in the case of nerve, passes through a super-normal phase before it returns to normal. Directly after the contraction, the excitability is so depressed that a very strong stimulus is required to produce excitation, but the strength necessary for this purpose gradually diminishes as the excitability returns. We may thus distinguish two phases: (*a*) an *absolute* refractory period during which no stimulus, however strong, can produce excitation; (*b*) a *relative* refractory period, during which

* ALISON DALE. *Journ. Physiol.*, 1932, 75, 1.

a second contraction is evoked by making the stimulus strong enough. The relation of these two phases in the excitability of cardiac muscle to the contraction produced by a single stimulus is shown in the accompanying diagram (Fig. 334). Directly after an effective stimulus is applied, the irritability of the preparation drops to zero and remains at this level during the phase of contraction. As relaxation of the cardiac muscle begins, excitability commences to rise, but some time elapses before it reaches normal. During this relative refractory period a second contraction of the muscle can be induced, the stimulus required becoming less as the absolute refractory period is left behind.

A chemical explanation would seem to be applicable to these facts. Heart muscle, like skeletal muscle, contains adenyl pyrophosphate and creatine phosphate. As with skeletal muscle, part of these are broken down when contraction occurs, and probably provide the energy for the contraction.* After the contraction the material is resynthesised. We may suppose that, just after the sudden breakdown of the labile substances has taken place, any further breakdown becomes for a time impossible; this time would be that of the absolute refractory period. As the store of labile material is regenerated, however, the excitability and contractility begin to return again, so that the tissue can respond to feebler and feebler stimuli, and at a certain point is great enough to enable the heart to respond to its normal "spontaneous" stimulus to contract. Just before this spontaneous contraction the heart muscle would respond to a minimal stimulus.

We should therefore expect the length of the refractory period to depend on the duration of the excitatory process: the smaller the breakdown as the result of excitation, the shorter will be the subsequent absolute refractory period. A contraction excited during the relative refractory period will be shorter than normal, because there has been less time for the accumulation of irritable material. Hence if the heart is stimulated to beat more frequently, the absolute refractory period following each beat is shortened, and we find that the length of the refractory period is inversely proportional to the frequency of the beat.

Thus Lewis found the following duration of the absolute refractory phase in mammalian auricular muscle under different rates of excitation :—

Rate per sec.	Refractory phase duration.
100	0·2 sec.
130–140	0·15 to 0·17 sec.
290	0·08 to 0·11 sec.

The refractory period is also shortened by stimulation of the vagus. This also will have the effect of diminishing the extent of the discharge at each beat. Thus the length of the refractory period seems to be determined by the amount of energy liberated or by the extent of the breakdown of irritable material in the previous contraction.

Under certain conditions the refractory period appears to be abnormally prolonged owing to the fact that a contraction is initiated by the second stimulus, but does not spread to the whole of the muscle, and hence is concealed (Drury and Love). This increase of the *effective* refractory period is seen when the muscle is subjected to cold, pressure, acid solutions, veratrine or quinidine, some of which, *e.g.* veratrine, do not alter the absolute refractory period at all, while others, *e.g.* quinidine, increase the absolute refractory period, but not to such an extent as might be supposed from estimations of the effective refractory period.

PREMATURE CONTRACTIONS. When a tracing is being taken from the ventricle, which is beating at its normal rhythm in consequence of the stimuli communicated to it from the sinus venosus, it is found that the application of a single shock to the ventricle early in its diastole, is followed by the appearance of a ventricular contraction some while before the normal one would be due. It is called a *premature contraction* (or, less accurately, an

* CLARK, EGGLETON and EGGLETON. *J. Physiol*, 1932, **75**, 332.

'extra systole'), and is followed by a 'compensatory pause' (Fig. 335). This is due to the fact that one of the impulses travelling from the sinus arrives at the ventricle during the refractory period ensuing on the application of the artificial stimulus; hence it produces no effect and the ventricle has to wait for the arrival of the next succeeding excitatory wave from the sinus before it gives its next beat. In certain cases the first

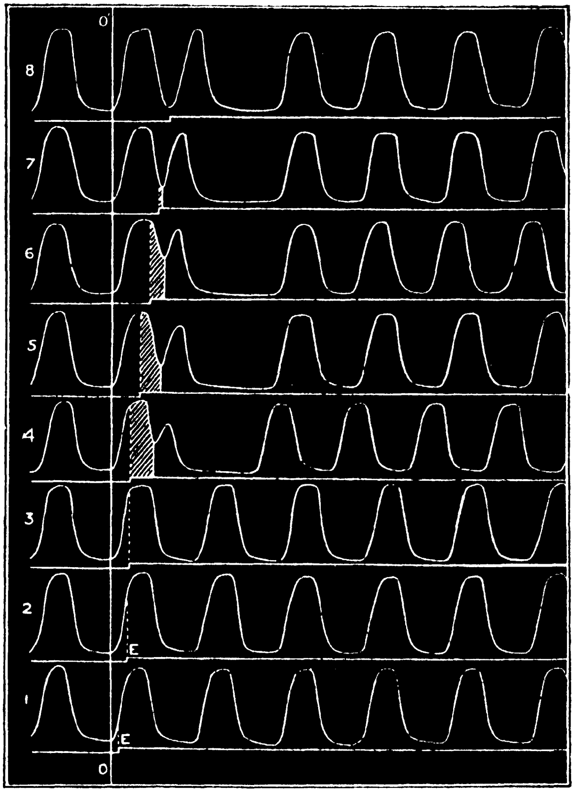

FIG. 335. Tracings of Spontaneous Contractions of Frog's Ventricle, to show Refractory Period. (MAREY, *La méthode graphique*, 1885, p. 417 (Paris, Masson.).)
In each series the ventricle was stimulated by an induction shock at E, as indicated by the tracing of the signal. In 1, 2 and 3, this stimulus had absolutely no effect, since it fell during the refractory period. In 4, 5, 6, 7 the effect of the shock was to interpolate a premature contraction in the series, the latent period (shaded part) gradually diminishing from 4 to 7. In 8 the irritability of the preparation was already considerable, and the latent period inappreciable. The 'compensatory pause' after the premature contraction is also well shown in 4, 5, 6, 7, 8.

contraction after the pause is considerably augmented. The compensatory pause, of course, does not occur when we are testing the effects of artificial stimuli on the sinus venosus.

INVERTEBRATE HEARTS. The hearts of invertebrates are often very different in structure and properties from those of the higher animals. In *Helix* the heart is composed of plain muscle, while in *Limulus*, which has been extensively studied by Carlson,* the heart forms a segmented tube of ordinary striated muscular fibres, which

* CARLSON. *Amer. J. Physiol.*, 1904, 12, 67.

has a local system of ganglion cells, but so situated that they can be cut away entirely from the muscular portions of the organ. Extirpation of the nerve cord abolishes spontaneous contractions. It is possible to make a nerve-muscle preparation of the anterior part of the heart, consisting of the muscle of the first two segments with a longer stretch of the lateral nerves. Stimulation of the lateral nerves with a single shock causes a single beat of the anterior segments; tetanising shocks cause a tetanus. The beat of the heart is originated and co-ordinated by the action of the local ganglionic centres. It would, for these and similar reasons, be wrong to transfer the results obtained on the heart of Limulus, or other invertebrates, to the explanation of the phenomena exhibited by the hearts of vertebrates.

THE MAMMALIAN HEART

In the mammal each side of the heart consists of an auricle into which the veins open, and a ventricle which receives the blood from the auricle

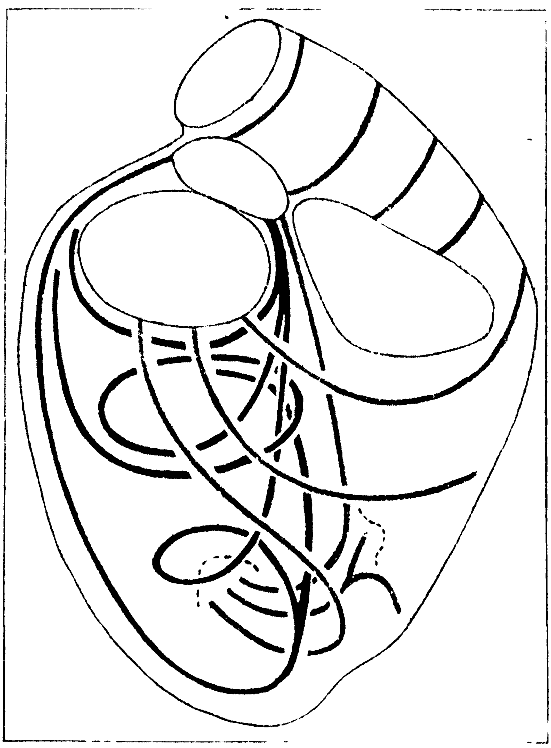

FIG. 336. View of the Heart from behind, to show the Course of the Chief Strands of Muscle Fibres. (MALL.)
The black lines represent the *bulbo-spiral* fibres, the grey lines the *sino-spiral* fibres.

and discharges it into the arterial trunk—either aorta or pulmonary artery. Since the auricles have to act merely as a receptacle for *part* of the blood which enters during the relaxation or diastole of the heart, their cavities are smaller than those of the ventricles, and their walls are thin, corresponding to the small amount of work thrown on them in propelling blood into the relaxed ventricle. The ventricles have the office of carrying on the main work of the circulation, viz. of forcing blood through the peripheral resistance. Their walls are much thicker than those of the auricles. The right ventricle has a wall which is only about one-fourth the thickness of the left ventricle,

in conformity with the much heavier work to be done by the latter. The capacity of both ventricles is approximately equal.

The auricles are separated from the ventricles by a fibro-tendinous ring. From this ring take origin most of the muscular fibres of the heart walls.

The muscular fibres of the auricles run in both circular and longitudinal directions (Fig. 336), the circular fibres being continued round both auricles, and special rings of circular fibres surrounding the openings of the great veins. From the fibro-tendinous ring between the auricle and the left ventricle and from the sides of the aorta, the muscular

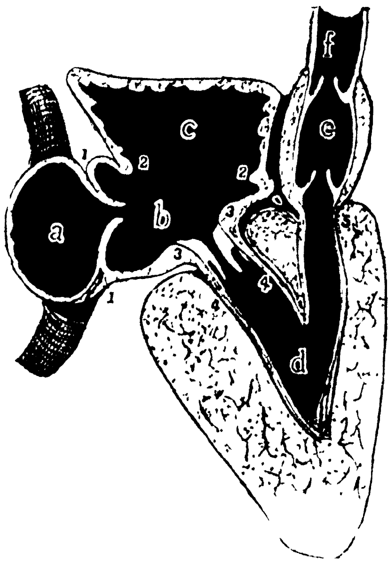

FIG. 337. A Generalised Type of Vertebrate Heart. (KEITH.)

a. Sinus venosus.
b. Auricular canal.
c. Auricle.
d. Ventricle.
e. Bulbus cordis.
f. Aorta.
1–1. Sino-auricular junction and venous valves.
2–2. Canalo-auricular junction.
3–3. Annular part of auricle.
4–4. Invaginated part of auricle.
5. Bulbo-ventricular junction.

fibres forming the superficial layer of the ventricular wall pass obliquely downwards to the left towards the apex of the ventricle. Here they loop round into the interior of the ventricle and pass up near its inner surface to end either in the papillary muscles or in the auriculo-ventricular ring of fibrous tissue. Between these two layers we find a third median layer of muscular fibres which is in the form of a muscular cone. The fibres of this layer form complete loops round the left ventricle. The middle layer is connected by many strands of muscular fibres with both inner and outer layers.*

As Harvey showed, the normal direction of the blood flow through the heart is determined mainly by the valves. The auriculo-ventricular valves are membranes attached round the entire circumference of the auriculo-

* *v.* LOWER. *Tractatus de Corde*, London, 1669, p. 24. (Facsimile and translation by Franklin, Oxford, 1932.)

ventricular ring (Fig. 338). They are composed of fibrous and elastic tissue covered on each side with endocardium, and project downwards into the cavities of the ventricles. On each side the membrane is divided by deep incisions into large flaps, three in number on the right side (the tricuspid valves) and two in number on the left side (the mitral valves.) The sail-like margins of these valves are connected by thin tendinous cords to the papillary muscles, which are projections of the muscular walls of the ventricles. By

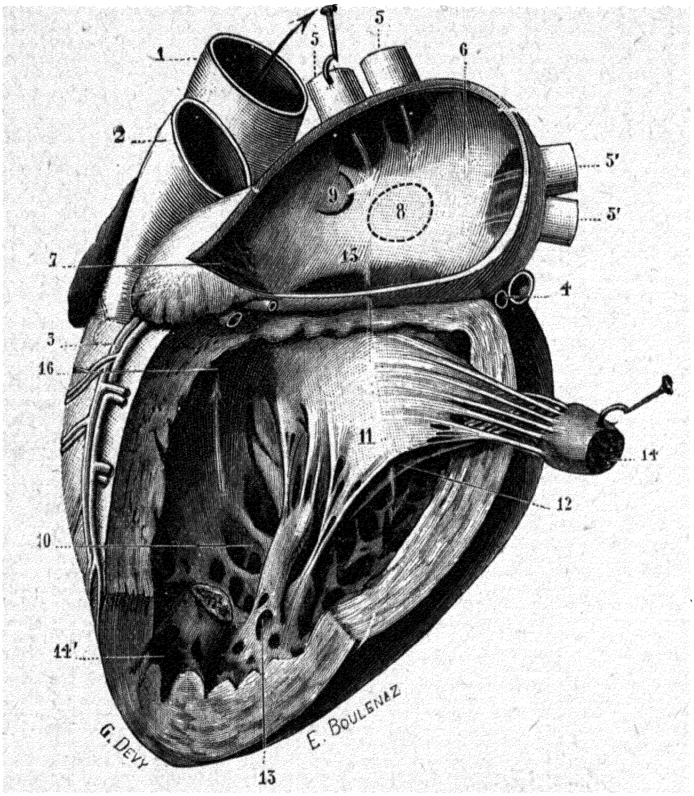

FIG. 338. Left Auricle and Ventricle, with Outer Side cut away to show Chief Points in Anatomy of Heart. (TESTUT.)

1. Aorta.
2. Pulmonary artery.
3. Ant. coronary vessels.
5, 5'. Pulmonary veins.
6. Left auricle.
7. Auricular appendage.
10. Cavity of left ventricle.
11, 12. Mitral valves
13, 14. Papillary muscles.
16. Arrow pointing to aortic orifice.

this means the edges of the valves are kept close together and prevented from eversion under the strong pressure exerted by the blood in the contracting ventricle. By the downward pull of the papillary muscles on the valves during the contraction of the ventricles, closure is rendered more complete, the inner surface of the valves being apposed over a considerable area. The action of the valves is aided by the contraction of the fibres surrounding the base of the heart, so that the auriculo-ventricular orifice is much smaller during systole than during diastole.

Each arterial orifice is provided with three valves, semilunar in shape and each attached by its convex borders to the arterial wall, and presenting

in the middle of its free border a small nodule, the *corpus Arantii*, from which fine elastic fibres pass to all parts of the valve. The extreme margin of the valve, the *lunula* on each side of the corpus Arantii is very thin. Whenever the pressure in the arteries is greater than that in the ventricles, these valves are closed, and the thin margins come in contact with similar portions of the adjacent valves, so preventing any reflux of blood. The borders of the valves under these circumstances come together in the form of a star composed of three lines at angles of 120°, the three corpora Arantii being pressed together at the centre of the star.

No valves are found at the orifices of the great veins into the auricles, and so a reflux of blood occurs during contraction of the heart, but it is limited by the contraction of the muscular rings round the veins, which slightly precedes the main auricular contraction.

The heart and the roots of the great vessels lie almost free inside the pericardium. This is attached below to the central tendon of the diaphragm, and above to the arterial trunks. It is lined by a layer of mesothelium continuous with a similar layer covering the surface of the heart. The two surfaces are kept continually moist by the pericardial fluid, so that the heart can move freely within the pericardium without friction. One of the chief functions of the pericardium appears to be to check an excessive dilatation of the heart.

THE CONTRACTION OF THE MAMMALIAN HEART

A perfectly rhythmic activity of the mammalian heart may be maintained for hours after its removal from the body by perfusing the coronary arteries with blood, or with a suitable warm oxygenated perfusion fluid.

It is possible to sever all the nervous and muscular connections between auricles and ventricles without stopping the contractions of the ventricles which, after a pause, recommence beating at a rhythm which is independent of and slower than that of the auricles. A piece of an auricular appendix, suspended in warm oxygenated Ringer's solution, will continue to beat for hours, and a fragment of the ventricular wall, free from ganglion cells, will similarly contract if fed by an artificial circulation through a branch of the coronary artery. We may therefore conclude that in the mammalian as in the amphibian heart, every part of the heart muscle possesses the power of rhythmic activity, the normal sequence of the beats being determined by the greater frequency of the natural rhythm at the venous end of the heart.

Conduction of Excitatory Wave. The excitatory condition started at one point in the muscle spreads through it in all directions, and the spread is independent of nerve fibres. As in the frog, the excitatory process may, in exceptional circumstances, be made to travel in a direction the reverse of normal, as, for instance, when the ventricles are excited at a rhythm of higher frequency than the natural beat which is starting at the venous end of the heart.* The phenomena are easily explained on the assumption that the whole of the musculature of the heart acts in many respects as a single muscle fibre, along which an excitatory process may be propagated in any direction. But in the mammalian heart the muscle fibres of both auricles and ventricles are seen to arise from a fibro-tendinous ring at the auriculo-ventricular junction, leaving apparently no muscular

* McWilliam. *J. Physiol.*, 1888, **9**, 167.

continuity between the two cavities. The discovery by Kent,* and His † of a distinct band of modified muscle fibres passing, in the septum, from the auricles to the ventricles, the '*auriculo-ventricular bundle*,' furnished an anatomical basis for the conduction of the wave.‡

In the adult heart, *e.g.* of man, the anatomical relations of the different cavities have become considerably modified from their primitive form (Fig. 337) in the course

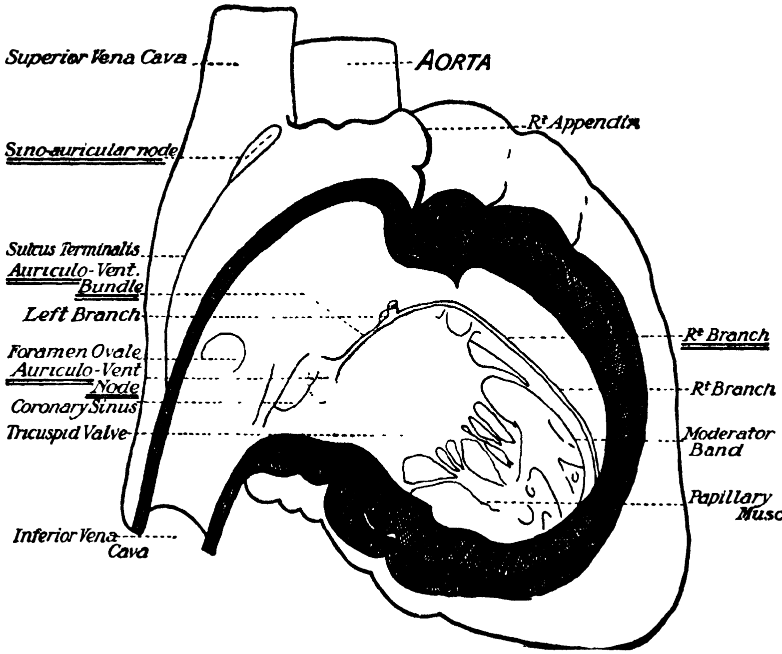

FIG. 339. Diagrammatic Representation of Course of A.V. Bundle.

of development. The sinus venosus, *i.e.* the part where in the lower vertebrates the contraction wave takes its origin, is now represented merely by the termination of the superior vena cava and of the coronary sinus in the right auricle in a small amount of tissue underlying the *sulcus terminalis* of the right auricle, as well as by the remains of the Eustachian and venous valves. The auricular canal gives rise to the auricular septum and to the auricular ring surrounding the auriculo-ventricular orifice, and in some hearts it is prolonged into the ventricle as the intraventricular or invaginated part of the auricular canal.

In the adult heart two accumulations of more primitive tissue are found in the region corresponding to the sinus venosus of the embryo, and these are known as the *sino-auricular node* § and the *auriculo-ventricular node*. They are composed of delicate interlacing fusiform fibres, faintly striated and embedded in dense connective tissue. The fibres contain very little glycogen. The sino-auricular node (Fig. 339) lies in the groove between the superior vena cava and the right auricle.|| The auriculo-ventricular node lies at the base of the auricular septum on the right side, below and to the right of the opening

* KENT. *J. Physiol.*, 1892, **14**, xxiii; 1893, **14**, 233.
† HIS. *Arbeiten. aus der med. Klin.*, 1893, **14** (Leipzig).
‡ TAWARA. "Das Reizleitungssystem des Saugetierherzens," Jena, 1908; TODD. *Johns Hopkins Hosp. Rep.*, 1919, **19**, 1.
§ KEITH and FLACK. *J. Anat. and Physiol.*, 1907, **41**, 172.
|| BLAIR and DAVIES. *J. Anat.*, 1935, **69**, 303.

of the coronary sinus. From this point a bundle of muscular fibres (the *bundle of His*, or the auriculo-ventricular or A.V. bundle) runs along the top of the interventricular septum just below its membranous part and then divides into the right and left septal divisions, which pass down in each ventricle on the interventricular septum into the papillary muscle arising from the septum. Each half of the bundle gives off several branches which break up, finally forming a reticulated sheet of tissue over the greater part of the interior of the ventricles just below the endocardium with branches that penetrate to end beneath the epicardium (Figs. 339 and 340). The fibres composing this tissue are more primitive in character than the rest of the cardiac musculature and have long been distinguished as the 'fibres of

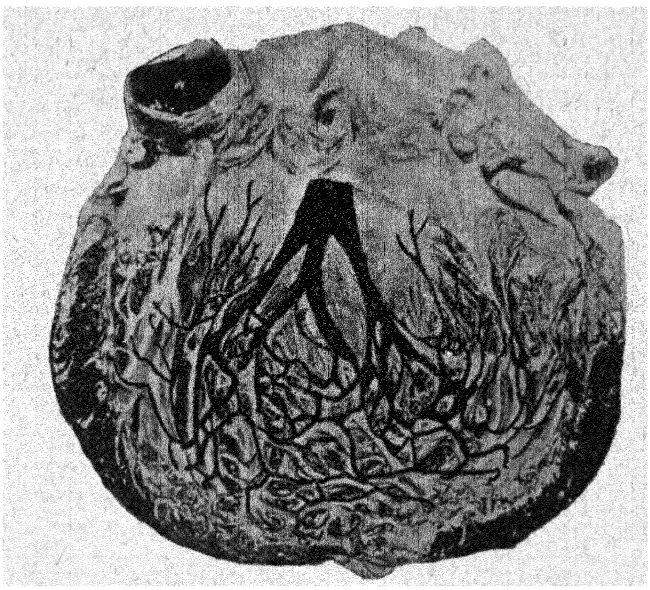

FIG 340. Left Ventricle, laid open to display the Interventricular Septum, on which the Course of the Left Division of the Auriculo-ventricular Bundle and its Ramifications are shown in black. (After TAWARA.)

Purkinje.' In them the fibrillation is confined to the periphery of the muscle cell (Fig. 341). They are distinguished by a high glycogen content. The course of the system of Purkinje tissue in the heart may be demonstrated by the injection of coloured materials into the sheaths which enclose the main strands.*

There are rich plexuses of nerves on the outer surface of the heart and also beneath the endocardium, and ganglion cells are freely scattered around the openings of the great veins, along the border of the interauricular septum, in the auriculo-ventricular groove and in the basal parts of the ventricles.

Numerous nerve fibres and ganglion cells are found to accompany the muscle fibres of the auriculo-ventricular bundle. We have, however, no reasons for regarding the nervous structures as concerned in the initiation or propagation of the excitatory wave.

Since the auriculo-ventricular bundle forms the only continuous muscular tissue between the auricles and ventricles, destruction of it causes complete abolition of the normal sequence of beat between auricles and ventricles.

* LHAMON. *Amer. J. Anat.*, 1912, **13**, 55; COHN. *Heart*, 1912, **4**, 225

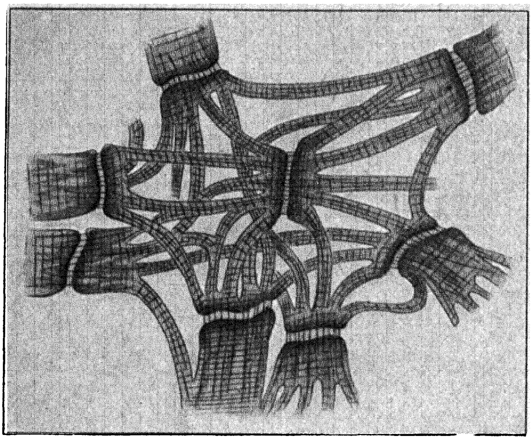

FIG. 341. Fibres of Purkinje, from the Sub-endocardial Network. (TAWARA.)

By leading off different parts of the heart to the string galvanometer, it is possible to determine the time relations of the excitatory process. It is then found that the sino-auricular node is the starting-point of the excitatory process concerned in each heart beat. It is therefore spoken of as the '*pace-maker*' of the heart. At each beat a contraction starts at the sino-auricular node, spreads a short way up the great veins, and along the auricular muscle in all directions (Fig. 342). When it arrives at the auriculo-ventricular node, the impulse is carried on to the ventricles along the auriculo-ventricular bundle, spreading along the branches of this bundle to almost all parts of the ventricular muscle. Although normally the sino-auricular node initiates each heart beat, this node can be put out of action by injury or great cooling without stopping the rhythmic sequence of the heart beat, the office of pace-maker being now taken up by the auriculo-ventricular node. A specialisation of function accompanies the differentiation in structure which we find in the auriculo-ventricular bundle and its branches. Lewis has shown that the conduction of the excitatory process along the auriculo-ventricular bundle of Purkinje tissue occurs about ten times as fast as the conduction through the ordinary muscular tissue of the heart, the rates being about 5000 mm. and 500 mm. per second respectively.

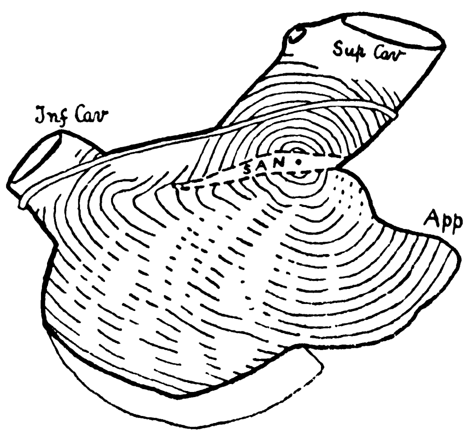

FIG 342. Diagram showing the manner in which the Excitatory Process spreads from the Sino-auricular Node over the Auricles and along the great Veins. (T LEWIS.)

The limitation of the muscular continuity to a single narrow bundle, which is endowed with greatly increased conducting powers and ends in a network of similar tissue, ensures that all parts of the ventricles contract practically simultaneously. If this were not the case, the sudden contraction of the muscle fibres near the base of the ventricles would simply bulge out the still uncontracted portion near the apex, and there would be a risk of injury of the uncontracted part of the ventricle.

Although all parts of the ventricular surface receive the impulse to contract almost simultaneously, *i.e.* within 8 msec. of one another in the dog's heart, the whole ventricles being involved within 0·07 sec., the contraction

wave is found to commence 10–17 msec. earlier at the surface overlying the interventricular septum. On the other hand, the conus of the pulmonary artery is the last part of the heart to begin contracting.

The conductivity of the auriculo-ventricular bundle may be impaired by disease. In such cases we get a series of phenomena known as *heart block*, the main characteristic of which is the slow contraction of the ventricle, accompanied by a rapid auricular wave in the venous pulse at a rhythm entirely independent of the ventricular pulse. The automatic activities of auricle and ventricle are, in fact, dissociated. At certain intervals or at certain stages of the disease, the fibres of the bundle may present only a partial block, so that the ventricle responds once to every second contraction of the auricle. The existence of this disease is shown at once on the electrocardiogram

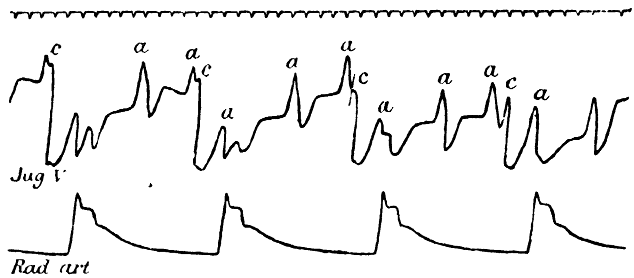

FIG. 343 Simultaneous Tracings of the Jugular Venous Pulse and the Radial Arterial Pulse, from a case in which the A V bundle was destroyed by disease. The contractions of the auricles are marked by the *a* waves on the venous pulse They are more rapid than, and quite independent of, the ventricular contractions (MACKENZIE)

(or venous pulse, see Fig. 343) by the dissociation of the normal relation between the auricular and ventricular components.

FIBRILLATION. The long refractory period in heart muscle renders it impossible to throw it into a tetanus. If, however, a strong faradic stimulus is applied during the latter part of systole to the ventricle of a heart beating normally, a premature contraction results and then the rhythmic contraction of the chamber stimulated ceases instantaneously; the chamber dilates and its walls are seen to be the seat of vermicular movements, each muscle fibre or fasciculus seeming to be contracting independently of the fibres around it, so that the whole surface is affected with a writhing movement.* The same condition of fibrillation may sometimes be produced in the mammalian ventricle by obstruction of one coronary artery, and may account for the sudden death which frequently occurs as a result of disease of the coronary arteries. It seems probable that a similar condition is sometimes responsible for the sudden death which may occur during the induction of chloroform anæsthesia.†

When fibrillation is induced in the auricles, it often passes off after a variable time and the ordinary rhythmic contractions recommence. Fibrillation of the auricles is a frequent complication of heart disease, where the myocardium has been injured by some previous infective process, such as rheumatic fever. The fibrillation is at the rate of about 450 per minute, and does not spread to the ventricles, but sets up frequent and irregular excitations which travel along the A.V. bundle and cause a rapid and irregular ventricular rhythm. The smaller contractions in this rhythm may be ineffective for the expulsion of blood from the ventricles, and merely promote the exhaustion of the heart.

* WIGGERS and WÉGRIA. *Amer J. Physiol.*, 1940, **128**, 500.
† LEVY. *J. Physiol.*, 1911, **42**, Proc. iii, **43**, Proc. xviii.

In some instances the auricular fibrillation is not general, and the effect of the local fibrillation is to impart a very rapid, but regular, rhythm to the auricles, while the ventricles have a regular rate one-half or a quarter that of the auricles. This is called *auricular flutter.*

Fibrillation in the ventricles is usually irreversible, and causes death.

The clue to the causation of fibrillation was given by observations of Mines * and of Garrey. Mines found that under certain conditions a ring of frog's ventricular muscle might respond to a single stimulus by a wave of contraction passing continuously round the ring, and Garrey observed the same phenomenon in a ring cut from the contractile tissue of the umbrella of the jelly fish. The way in which this circus movement is brought about has been elucidated by T. Lewis. In Fig. 344 is represented a ring of auricular tissue. If it is excited rhythmically at X, the wave of excitation will travel in both directions round the ring and finish at Y. If, however, the rate of stimulation be continually increased, a time will arrive at which any slight difference in the duration of the refractory stage at *a* and at *b* becomes important. Let us suppose it is shorter at *b*. The wave due to the second stimulus may therefore be able to pass *b* but not *a*. The wave will thus pass in one direction only (anti-clockwise), and will travel right round the ring. By the time it has travelled past Y and arrived at *a*, the refractory stage may have passed off, so that the muscle at *a* can now contract. Whether the circus movement continues indefinitely will depend on (1) the rate of conduction, (2) the length of the ring, and (3) the duration of the refractory period. Anything which diminishes the rate of conduction and the duration of the refractory period (and both these changes result from frequent stimulation) will tend to set up a circus movement. When fibrillation is present, there is a circus movement in a ring of tissue round the mouths of the great veins. This ring is not, however, separated from the rest of the auricle, so that the wave of excitation in its course round the ring will excite in turn the adjacent portions of the auricular muscle, so that these will contract at all intervals after the passage of the wave past X. All co-ordinate movements will then be abolished and the auricle will continue in a state of fibrillation. This explanation is supported by the fact that it is possible to set the auricle into fibrillation by the application of two shocks at such an interval that the second falls just outside the absolute refractory period of the auricular muscle, and the same thing doubtless occurs when the auricle or ventricle is set into fibrillation by the application of strong faradic currents. Administration of quinidine puts a stop to auricular fibrillation in about 50 per cent of cases, and this is owing to the fact that it increases the effective refractory period.

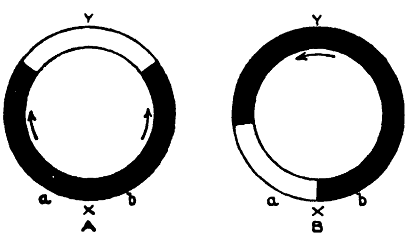

FIG. 344.

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THE SEQUENCE OF EVENTS IN THE CARDIAC CYCLE

The heart beats about seventy times a minute in normal resting human adults. Each beat begins by a simultaneous contraction of both auricles, and of the auricular appendages, which become pale and bloodless. The contraction of the auricles is followed by that of the ventricles, as a result of which blood is thrown out into the large arteries. In clinical terminology, the term 'systolic' refers to the ventricular systole, so that '*pre-systolic*' events occur during auricular systole. The duration of ventricular systole in man is given by the interval between the commencement of the first and second heart sounds, or by the interval between the upstroke of the R nerve and end of T nerve on the electrocardiogram. The contraction of the auricles lasts about a tenth of a second, that of the ventricles about three-tenths of a second. The period of total relaxation or diastole lasts about four-tenths of a second. During this cycle of changes the following events are taking place within the heart:

In the diastolic period the aortic valves are closed, and the auriculo-ventricular valves open. There is a continuous flow of blood from veins, through auricles into ventricles and, as the walls of both these cavities are relaxed, there is no impediment to the inflow of the blood until the heart is quite full (period of diastasis) and begins to stretch the pericardium. Under normal circumstances the diastole comes to an end before such restraining influence of the pericardium can be effective.

The contraction of the auricles drives some of their contents into the ventricles and so still further increases their filling, no resistance being offered by the flaccid wall of the ventricles. The amount of blood propelled into the ventricle by the auricular contraction has been variously estimated at from 18 to 60 per cent. of the total ventricular filling. In fact it is a variable quantity, depending on complex dynamical factors, chief of which is the time relation between auricular and ventricular systoles.* But even when the auricle is fibrillating, and non-functional, ventricular filling may still be adequate. As the blood rushes from auricle into ventricle through the funnel-shaped opening of the membranous tube formed by the valves, eddies are set up in the ventricle tending to close the valves, so that they are held, by the resultant of the two opposing currents, in a condition midway between closure and opening.

The onset of the ventricular contraction is extremely sudden. There is a quick rise of pressure in the ventricle, which presses together the flaps of the mitral or tricuspid valve, while the bases of these valves are approximated by the contraction of the circular fibres at the base of the ventricles. As the heart shortens in systole the papillary muscles also shorten, so that the valves are prevented from being everted into the auricles, while the blood is pressed, so to speak, between the cone of the ventricular wall and the cone formed by the closed valves.

The outflow of blood from the ventricles, however, does not commence instantly, and for a very short time, therefore, the ventricle is contracting on a closed cavity, and is thus performing an *isometric* contraction (isometric phase), during which the pressure within it must rise abruptly. Before

* JOCHIM. *Amer. J. Physiol.*, 1938, **122**, 639.

the beginning of systole the pressure in the ventricular cavity is quite small (only 2 or 3 mm. Hg), while the pressure in the aorta is 70 to 90 mm. Hg. Before the aortic valves can be opened, the pressure in the left ventricle must rise to a point which is greater than that in the aorta, and similarly on the right side of the heart. As soon as this happens the valves open and the outflow of blood (ejection phase) commences, and continues so long as the pressure in the ventricles is higher than that in the great arteries.

Even in extreme contraction the cavities of the ventricles are never entirely obliterated, though the right ventricle is reduced to a narrow slit widening out slightly in the neighbourhood of the auriculo-ventricular orifices, while in the left ventricle a distinct cavity is left between the mitral valves and the free ends of the papillary muscles. During normal activity the emptying of the cavities rarely proceeds to so great an extent. Directly the ventricular pressure falls below the arterial pressure, the arterial valves must close again and the output of blood come to an end.

In order to obtain an accurate idea of the progress and duration of each of these events in the cardiac cycle, it is necessary to study the changes occurring in the pressure within the auricles and ventricles during the various phases of the heart beat.

THE ENDOCARDIAC PRESSURE. A manometer which shall register accurately the changes in the pressure within the heart must be capable of responding to very rapid changes. Thus in the left ventricle at the beginning of the systole, there may be a rise of 130 mm. Hg in 0·06 sec., *i.e.* at the rate of nearly 3 atmospheres per sec. In a heart beating rapidly and forcibly under the action of adrenaline, the rise may be still more rapid, *e.g.* 8 atm./sec. A mercurial manometer would be quite unequal to registering such rapid changes of pressure. We require an instrument to possess small inertia and be capable of registering a rapid rise of pressure without entering into oscillations of its own.

The membrane manometer of Hurthle is a small chamber with a stout rubber top, entirely filled with fluid, and connected with the cavity of the heart by a tube also filled with fluid (sodium sulphate solution) and passed through the jugular vein or carotid artery, or better, thrust directly through the wall of the ventricle.

Even Hurthle's instrument is inadequate to give a correct representation of the most rapid changes of pressure occurring in the contracting ventricle. A study of the theory of recording instruments by Otto Frank * showed the fundamental requirements of such an instrument were that the mass of fluid and of parts of the instrument moved must be as small as possible. Moreover, in order to avoid vibrations of instrumental origin, the period of self-vibration of the mass moved must be as high as possible. This is accomplished, as in Hürthle's instrument, by using a very small tambour, covered with a strong, tightly stretched membrane connected, by as short and wide a tube as is feasible, with the heart or blood vessel where it is desired to register changes of pressure. The whole is filled with fluid. In the best patterns the oscillations of the membrane are recorded by means of a beam of light which impinges on a mirror attached to the membrane and is reflected on to a moving photographic surface.

Manometers on these principles have been designed by Frank (Fig. 399, p. 640), Wiggers, and Piper. Wiggers' manometer † is shown in Fig. 345. At its lower end cannulæ of various forms and sizes can be attached. At the middle of the tube is a short lateral tube to which, by a ball and socket joint B, is attached a tiny 'segment capsule' A. Over the capsule is stretched a thick rubber membrane, to which on the straight side of the segment a small mirror is affixed. The entire

* Frank. *Z. f. Biol.*, 1908, **50**, 309; 1925, **82**, 49.
† Wiggers and Baker. *J. Lab. Clin. Med.*, 1924, **10**, 54.

capsule and its mirror can be adjusted in any direction by the handle D. A second small mirror E is carried on a small plate. This serves to project a base line, and is adjustable by the handle F. Alterations in the pressure within the manometer cause minute oscillations of the membrane, which can be recorded and magnified to any desired extent by means of a beam of light reflected from the mirror on to a moving photographic plate or paper (Fig. 346). The 'figure of merit' of this manometer, *i.e.* its own period of vibration, when filled with fluid, is about 450 per second with a thick membrane, so that it can record with accuracy such rapid changes of pressure as occur even in the left ventricle.

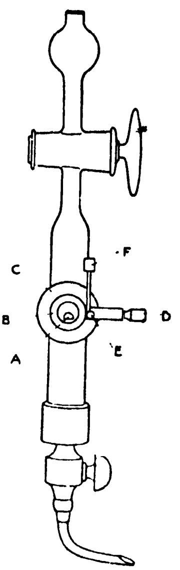

FIG. 345. Wiggers' Manometer, for Optical Recording of Blood Pressure (about one-third full size).

INTRAVENTRICULAR PRESSURE CHANGES. In order to interpret these curves, we must have a simultaneous record of the pressures in the auricle and ventricle, or in the ventricle and aorta. Fig. 347 represents curves obtained from the left ventricle and left auricle. Nearly always it is possible to see on the up-stroke of the ventricular curve one or two elevations, the most noticeable being the elevation marked S_1. This can be shown to correspond to the opening of the aortic valves.

The average course of the changes of pressure in the left heart during each beat is shown diagrammatically in Fig. 348. The cardiac cycle begins with the contraction of the auricle, which may cause a slight rise of pressure in the ventricle. As the auricular contraction dies away, the ventricular contraction begins at I. This causes a very rapid rise of pressure. Almost immediately after the beginning of the rise the auriculo-ventricular valves close, and the *isometric phase* begins. In this the pressure rises rapidly in the ventricular cavity without causing any change in its contents. Directly the pressure exceeds that in the aorta, the aortic valves open at the point marked II., where the *ejection phase* begins, and the aortic pressure then rises with the ventricular pressure. During the whole duration of this phase of the contraction, the aortic pressure remains somewhat below the ventricular pressure, showing that blood is flowing continuously from ventricle into aorta. The ejection of blood is at first rapid, and the pressure in the ventricles continues to rise. As the heart gets smaller, the amount of blood ejected into the aorta becomes less than that flowing out in the unit of time through the peripheral branches, so that the pressure

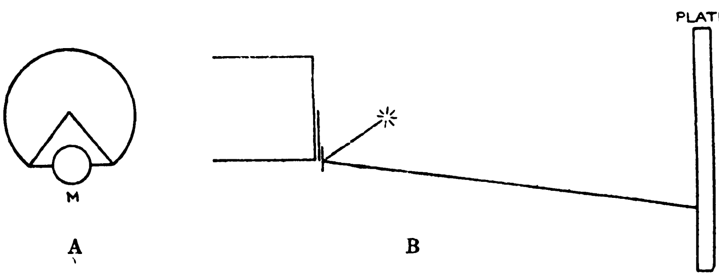

FIG. 346. A, Magnified Diagram of a 'Segment Recorder' covered with Membrane, to which is connected the minute Mirror M (front view).
B, Diagram showing how a Beam of Light is thrown on to the Mirror and then reflected on to a moving Photographic Plate.

begins to fall in the aorta and ventricle, even though an outflow of blood is still going on. The ejection period may therefore be divided into two phases, that of *maximum ejection*, and that of *reduced ejection*. Usually this portion of the curve forms a smooth arch, but under some conditions it may be flattened to form a plateau, of horizontal, ascending or descending slope. The ventricular muscle suddenly relaxes at the point marked IV., causing a sudden fall of pressure in the ventricle, and a slight fall in the aorta. This latter, however, is arrested almost at once by the closure of the aortic valves, marked by the

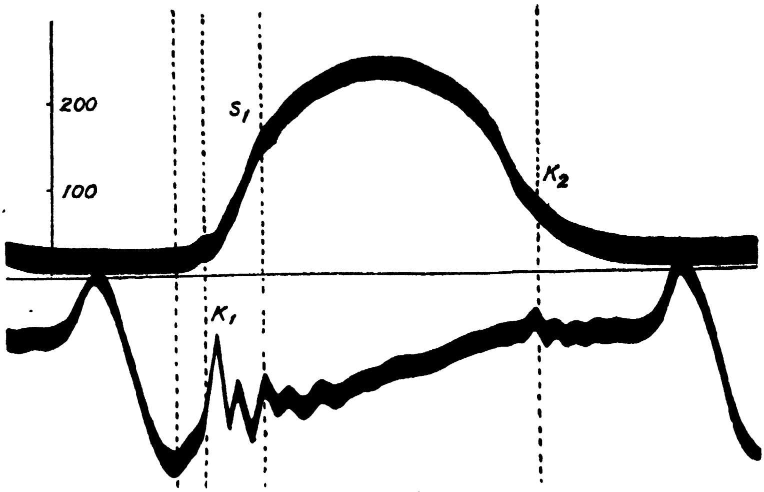

FIG. 347 Endocardiac Pressure Tracings, taken with Piper's Manometer.

Simultaneous tracings, the upper from left ventricle and the lower from left auricle. To be read from left to right.

K_1 Closure of A.V. valves. S_1 Opening of aortic valves.
K_2 Opening of A.V. valves.

sharp depression, the *incisura* (V), in the aortic tracing. Between V. and VI. the relaxation is isometric, since all the valves guarding the orifices of the ventricle are closed. The pressure in the ventricle continues to fall, until at the point VI. it drops below that in the auricle, and the auriculo-ventricular valves open, allowing the inflow of blood from pulmonary veins and auricle. The pressure in the ventricles then reaches the line of zero pressure, and remains at or near this line during the greater part of diastole. With a big inflow there may be a slight rise towards the end of diastole, which may be accentuated by the auricular contraction. If the chest is opened, the pressure in the ventricle never sinks below zero during any part of diastole. Naturally, under normal conditions, the pressure in the closed chest outside the heart is ' negative ' owing to the elastic retraction of the lungs, and may vary from — 3 to — 30 mm. Hg, according as it is measured during normal expiration or during forced inspiration. This negative pressure may be transmitted to the interior of the heart; hence, in the closed chest the pressure in the heart cavities during diastole may, as in Fig. 348, be negative, but never falls below the intra-pleural pressure which obtains outside them, *i.e.* the heart does not actively exercise suction during diastole.

The time relations of these events naturally varies with the frequency of contraction of the heart. In the human heart, beating 75 times per minute,

a cardiac cycle will last 0·8 seconds. The following may be taken as the average duration of the different phases in the left ventricle (Wiggers):—

(1) Last phase of ventricular diastole. A small rise of pressure due to contraction of the auricles, lasting 0·05 second. The whole auricular

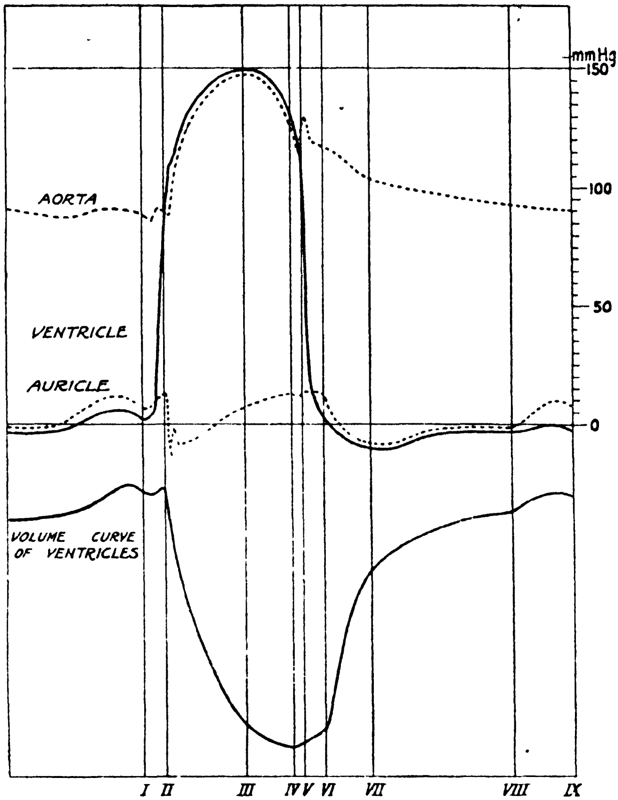

FIG. 348. Pressure Curves from Aorta, Ventricle and left Auricle, together with Volume Curve of the two Ventricles during one complete Cardiac Cycle. (Modified from WIGGERS.)

systole (rise and fall of intra-auricular pressure) takes about 0·11 second.

(2) The isometric phase, initiating systole, lasts 0·01 to 0·06 second. It varies but little with alteration in heart rate.

(3) The total duration of ventricular systole is about 0·3 second, varying from 0·33 to 0·23, as the heart rate quickens from 60 to 120 per minute.

(4) The diastolic phase may be divided into the following periods:—

(a) Protodiastolic phase (from beginning of relaxation to closure of aortic valves), 0·038 second.
(b) Isometric relaxation, 0·076 second.
(c) Rapid inflow phase, 0·113 second.
(d) Slow inflow phase, 0·163 second.

} Joint diastole = 0·39 sec.

The relative durations of these last two phases will naturally vary according to the rate of the heart and the amount of blood flowing into the heart in unit time.

The average relation of these various phases of the heart cycle are repre-

sented diagrammatically in Fig. 349. Similar but smaller pressure changes occur in the right side of the heart.

INTRA-AURICULAR PRESSURE CHANGES. Owing to the absence of valves between the right auricle and the venæ cavæ, changes of pressure within this cavity are transmitted along the veins. Observation shows that the jugular vein pulsates at least twice for each heart beat. The exact form of the pressure tracing in the auricles varies considerably, according to the inflow of blood and the state of filling of their cavities. A typical tracing with a moderate inflow of blood is given in Fig. 347, p. 580, and in this figure the relations of the different elevations in the auricular tracing to the intra-

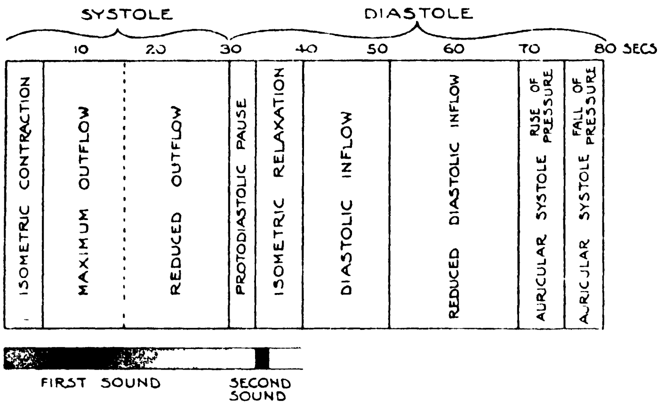

FIG. 349. Diagram showing average Time Relations of Events constituting a Cardiac Cycle.

ventricular events can be made out; in Fig. 350, the essential features of the curves are similar. In every case the auricular curve presents the following features:

(1) The first positive wave (pre-systolic wave) corresponding to the auricular systole.

(2) The second positive wave *k* (first systolic wave) occupying the beginning of the ventricular systole. This is caused by the sharp closure of the A.-V. valve.

(3) A negative wave of variable extent, largely due to the contracting ventricle pulling down the A.-V. ring towards the apex, and so opening out the auricle ' like a concertina.'

(4) A third positive wave (second systolic wave) which may present secondary undulations. This rise of pressure is due to the gradual filling of the auricles while the auriculo-ventricular valves are still shut.

(5) A negative wave, not always well marked. At this point (U.V., Fig. 350) the ventricle is entirely relaxed and the auriculo-ventricular valves open, so allowing the blood to flow freely from the auricle into the ventricle.

The pressure rises in the left auricle somewhat higher than in the right auricle. In the latter the big veins act as a supplementary reservoir to the auricle, so that in no period of the cardiac cycle need the pressure in the latter chamber rise to any extent. In auricular tracings the heart sounds are often apparent as small oscillations in the curve (Fig. 347).

CHANGES IN FORM OF THE HEART. Under normal circumstances the heart in the unopened chest lies in the pericardium, and is also supported laterally by the lungs, which have very little influence on its

shape. As the heart walls are flaccid during diastole, its shape will depend upon the position in which the heart is lying. Thus if the chest and the pericardium be opened and the animal be in the supine position, the heart during diastole will be flattened from before backwards. In this position, therefore, systole will be accompanied by a shortening in the lateral and vertical directions and a lengthening in the sagittal direction. During

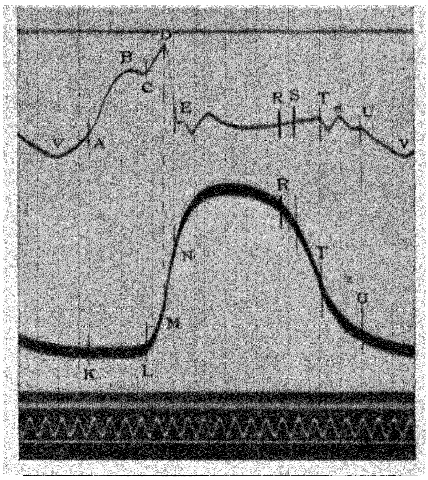

FIG. 350. Synchronous Left Auricular (upper) and Left Ventricular (lower) Pressure curves. (WIGGERS.)
A, B, C = Auricular systole. C, D = Second positive wave.
R = End of ventricular systole. U, V = Rapid inflow phase.
Time = 0·02 second.

systole the heart, whatever its previous condition, takes the form of a truncated cone.

When the heart is freed from the pericardium, the obliquity of its fibres causes the apex to move forwards and to the right during systole; this movement is normally prevented by the attachment of the pericardium to the central tendon of the diaphragm, so that the most movable part of the heart is then the base, which moves downwards towards the apex. This movement is determined partly by the shortening of the fibres of which the ventricular wall is composed, partly by the lengthening of the great arteries as blood is forced into them under pressure from the ventricles. It was observed by Leonardo da Vinci that if three needles be passed through the chest wall so that their points lie, one in the base, one about the middle of the ventricles, and one in the apex of the ventricles, each ventricular systole is accompanied by a downward movement of the needle in the base of the heart, a slighter movement of the needle in the middle of the ventricles, and practically no movement at all of the needle which is thrust into the apex.

The Cardiac Impulse. Each ventricular systole causes a throb to be communicated to the chest wall, over a limited area of which it may be felt and seen, except in fat individuals. The region where the pulsation is most marked usually lies in the fifth intercostal space, a little to the median side of the left nipple. This position may shift with alterations in the work of the heart, as after exercise. The pulsation is spoken of as the *cardiac impulse* or ‘apex beat,’ though the apex of the heart is situated lower down. During diastole the ventricles form a flabby flattened cone lying against the

chest wall and slightly deformed by the latter, especially when the subject leans forwards, or assumes the prone position. In systole the ventricles become rigid, assuming the form of a rounded cone. This sudden recovery of shape and hardening of the ventricular wall pushes out that part of the chest wall in immediate proximity to the ventricles, and so gives rise to the

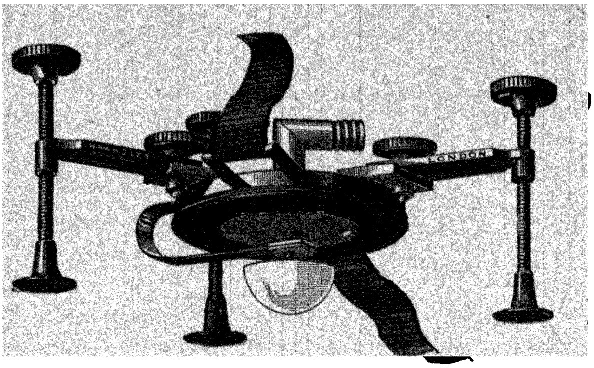

FIG. 351. A Cardiograph. The central button is applied to the 'apex beat, and its pressure on the chest wall regulated by means of the three screws at the sides. The tube serves to connect the cardiograph with a registering tambour.

cardiac impulse. The cardiac impulse, by its characters of force, regularity, requency and maintenance, gives valuable clinical information as to the occurrence and nature of the ventricular contraction.

The cardiac impulse may be registered by means of a cardiograph (Fig. 351).

The curves known as cardiograms may vary considerably in the same subject according to the pressure employed and the exact spot at which the tambour is applied. Their interpretation often presents difficulties owing to the fact that their form is conditioned by two factors, viz. (1) the actual size of the ventricles; (2) the tension of the ventricular wall. Fig. 352 represents a cardiogram which presents considerable similarities to the endocardiac pressure curve. The auricular systole finishes before the main rise of the lever occurs. In many cases, however, the elevation due to the auricular systole may take up the greater part of the ascending limb of the curve. The great variability in the type of curve makes the cardiogram of little value.

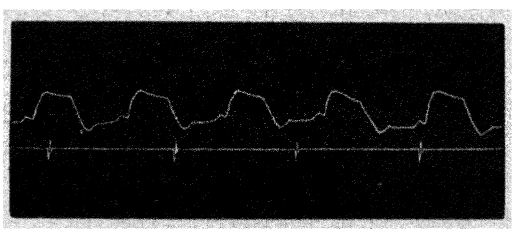

FIG. 352. Cardiogram. (HÜRTHLE.)

THE HEART SOUNDS. If we apply the ear, or listen with a stethoscope, to the front of a person's chest, we hear two distinct sounds accompanying each cardiac impulse, *i.e.* each systole, followed by a pause corresponding to the diastole. The sounds are often, though inadequately, compared to the syllables *lubb*, *dup*, the first sound being low-pitched and prolonged, the second sound high and sharp. Thus the heart sounds may be represented: 1st, 2nd, (pause), 1st, 2nd, (pause).

The *first sound* is best heard at the situation of the cardiac impulse. It is caused by the sudden closure of the auriculo-ventricular valves and the sudden increase in tension upon them and in the ventricular walls, whereby all these structures are caused to vibrate. It was formerly believed that the contraction of the ventricular muscle contributed a component to the first

sound, but any such component is probably small, because little sound is produced if means are adopted to prevent alteration in tension of the ventricular walls and of the auriculo-ventricular valves.* The first sound is reduplicated if the two ventricles contract asynchronously (as in bundle branch block).†

The *second sound* is heard most loudly just over the second right costal cartilage, *i.e.* where the aorta lies nearest the surface. It comes at the end of ventricular systole, as determined by the cardiac impulse, and is synchronous with the closure of the aortic valves. It is, in fact, caused by the vibrations set up in the valves, and in the walls of the aorta near them, consequent upon their sudden shutting and stretching occurring directly the heart ceases to contract and to force the blood into the aorta. If the valves be hooked back in an animal by means of a wire passed down a carotid artery, the second sound disappears and is replaced by a murmur caused by the blood rushing back into the ventricle at the end of the systole.

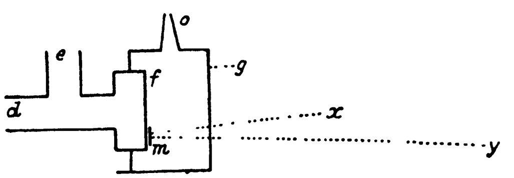

FIG. 353. Diagram showing construction of Wiggers' Capsule for the Direct Registration of Heart Sounds.

The pulmonary and aortic valves generally close simultaneously. In some cases, however, the aortic may close slightly before the pulmonary, giving rise to a 'reduplicated second sound.'

The distinctness with which the heart sounds are heard at the surface of the chest depends primarily on the physical conditions for conduction, such as the thickness of the chest wall, &c., but other things being equal, the loudness of the first sound depends on the abruptness and force with which the ventricle contracts, while the loudness of the second sound varies with the force with which the valve is closed.

THE GRAPHIC RECORD OF THE HEART SOUNDS. Various methods have been devised for graphically recording the heart sounds together with other occurrences, so as to display their relation to other events in the cardiac cycle. One method is to convey the heart sounds to a microphone, which is thus set into vibration. The alterations in current so produced are recorded by a string galvanometer. The sound vibrations may also be recorded directly, by receiving them on a membrane carrying a small mirror, the vibrations of which are recorded photographically. Wiggers' and Dean's‡ instrument for this purpose is shown diagrammatically in Fig. 353. The segment capsule *f* is connected by a tube *d* with the bell of a stethoscope applied to the chest. The side tube *e*, which is adjustable, is to prevent the gross movements of the chest (apex beat) being transmitted to the membrane. The membrane is a delicate rubber film, formed by dipping the end of the capsule into rubber cement, which is allowed to dry. To it is affixed a tiny mirror *m*. To protect this membrane from extraneous sound vibrations it is enclosed in a housing *g*, having a front window of glass and a conical vent *o*, which serves the same purpose as the Eustachian tube of the middle ear, permitting equalisation of pressures on the two sides of the membrane. A record of the heart sounds is shown in Fig. 354.

THE THIRD HEART SOUND. A third heart sound is described § as occurring in certain individuals during the diastole, shortly after the second sound. It may be

* ECKSTEIN. *Amer. J. Physiol.* 1937, **118**, 359; LEWIS and DOCK. *J. Amer. Med. Assoc.*, 1938, **110**, 271.

† BRAUN-MENÉNDEZ and SOLARI. *Arch. Int. Med.*, 1939, **63**, 830.

‡ WIGGERS and DEAN. *Amer. J. Med. Sci.*, 1917, **153**, 666; *Amer. J. Physiol.*, 1917, **42**, 476.

§ EINTHOVEN. *Pflüger's Arch.*, 1907, **120**, 31.

mistaken for a reduplicated second sound. It is softer and of a lower pitch than the second sound, and is heard most distinctly over the apex beat. It is probably due to the vibrations set up in the fluid itself, or in the auriculo-ventricular valves, by the

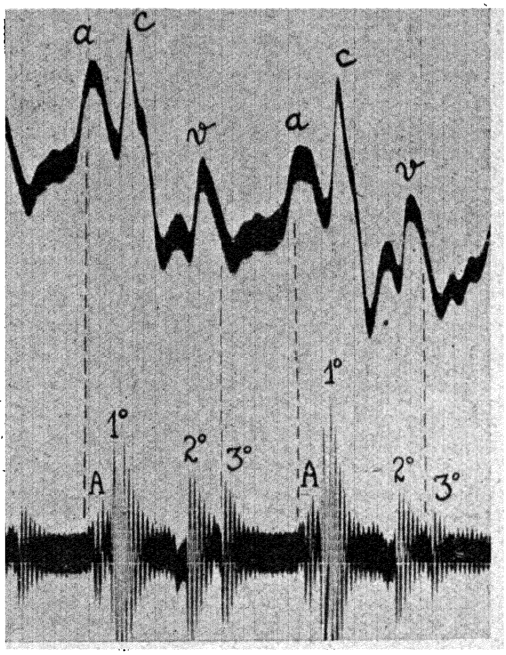

FIG. 354. Jugular vein pulse and heart sounds in man. In addition to the audible first and second sounds, the normally inaudible auricular sound (A) and the third sound are shown. (E BRAUN MENÉNDEZ and O. ORÍAS. *Rev. Argent. Cardiol*, 1934, 1, 101.)

sudden inrush of blood from auricles to ventricles at the beginning of the diastole.* The sound is shown in Fig. 354, as is also a very faint sound produced in auricular systole. Though not audible in normal hearts, both are detectable with delicate recording apparatus. This auricular sound is sometimes loud enough to be mistaken for a reduplicated first sound.

CARDIAC MURMURS. When a fluid escapes through a narrow orifice into a wider space, vibrations are set up in the fluid and may cause sound. The same sort of vibration may be set up in the large vessels or in the heart, whenever the blood passes rapidly through a narrow orifice into a wider space.

In the normal resting individual, sounds produced in this way, though detectable by delicate recorders, are so slight that they are inaudible; examples are seen in the third sound and in the auricular sound already mentioned; under abnormal conditions, as after diseases affecting the valvular orifices of the heart, or after exercise, they may be heard with ease. These murmurs, or *bruits* as they are called, are of paramount importance in enabling the medical man to form a judgment as to the condition of the different valves of the heart. Thus injury to an aortic valve, so as to allow of leakage during diastole, allows a small amount of fluid under high pressure to pass from the aorta into the relaxed ventricle. On listening to the chest of a man with such a lesion, this regurgitation during diastole is heard as

* GIBSON. *Lancet*, 1907 (ii), 1380.

a rushing sound occurring in the place of, or continuing, the second sound up to the beginning of the next first sound, which denotes the commencement of systole.

In many cases, the disease which occasions the inadequacy of the aortic valve is followed by cicatrisation, so that the orifices can never become thoroughly patent or properly closed. Under such circumstances vibrations will also be set up in the current of blood as it escapes through the narrow orifice into the aorta during systole; and on listening to the chest over the second right costal cartilage, a 'to and fro' bruit is heard composed of a systolic immediately followed by a diastolic murmur. In the same way, incompetence of the mitral valve, or dilatation of the mitral orifice in consequence of weakness of the cardiac muscle, gives rise to a murmur which lasts during the whole of the ventricular contraction and is therefore *systolic* in relation. Such a murmur is heard best over the area of the cardiac impulse, and is also transmitted backwards, so that it can be heard on listening at the back of the patient. A narrowing of the mitral orifice in consequence of contraction of the valves will set up a resistance to the flow of blood from left auricle to left ventricle. The auricle becomes hypertrophied, its contraction prolonged, and the passage of blood through the contracted orifice gives rise to an auricular murmur which is heard on listening over the apex beat as a *presystolic bruit*. This bruit is easily distinguished from a systolic murmur by noticing that it runs up to and ends with the cardiac impulse, whereas a systolic murmur does not begin until the cardiac impulse commences.

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THE HUMAN ELECTROCARDIOGRAM. When skeletal or other muscle is immersed in saline solutions, the electrical changes during excitation are not of the simple diphasic or monophasic nature obtained when the tissue

FIG. 355. Jugular venous pulse, radial arterial pulse and electrocardiogram in man. The jugular waves *a*, *c*, *v* are marked; also the E.C.G. waves P, R, T. Note that the P precedes *a*, and *c* precedes the radial upstroke. The interval between auricular and ventricular systole, as measured by *a*—*c*, is slightly greater than that obtained from P—R (0·15 secs.). The summit of R is about 0·03 sec. before ventricular systole begins; the summit of T is at height of systole and end of T about 0·03 sec. before second sound.

is in air.* Similarly, if we lead off any two parts of the heart's surface to a string galvanometer or capillary electrometer, we record potential changes which are caused, partly by events occurring in the muscle just underlying the electrode, partly by changes occurring at a distance and transmitted by the intervening muscle and blood acting simply as a moist conductor.† These two kinds of effect may be alluded to as direct and indirect. If we lead off, not from the heart itself, but from neighbouring tissues in contact with the heart, we shall still obtain the indirect effects of the electrical changes at each heart beat, and these can be obtained when the intact animal is led off to the galvanometer by his limbs. In man, where the heart lies asymmetrically, with its electrical axis varying during systole, but roughly lying in an oblique direction pointing downwards towards the left, it is usual to lead off the right arm and left arm (lead I), the right arm and left leg (lead II), the left arm and left leg (lead III), or the precordium and right arm (lead IV.R), or left leg (IV.F) by suitable electrodes, to a string galvanometer or cathode ray recorder (Fig. 85, p. 169). By this means an *electrocardiogram* is obtained similar to that shown in Fig. 355. The different points in a typical tracing are designated by the letters P, Q, R, S, T, first applied by Einthoven and retained because they do not commit us to theoretical interpretation of the curves, which, in view of the reasons given above, would be difficult. Such interpretation as we have is the result of direct experimental observation, which shows that the electrocardiogram is a composite picture of different events in the heart; that P is certainly due to the auricular excitation, and Q marks the beginning of the ventricular excitation. The P wave averages 0·08 sec. and precedes auricular contraction by 0·02 sec. Q, R, S varies normally, with heart rate, from 0·04–0·08 sec., and represents the spread of excitation in the ventricles. It precedes their contraction. Q—T (0·43 sec.) gives duration of systole. The A—V. interval (0·12–0·2 sec.) is given by the distance between P and R (0·12–0·2 sec.), the total duration of the excitatory condition in the ventricle by the distance between Q and T. The highest wave, R, ranges normally from 0·7 to 1·8 mv. The T wave (0·27 sec.) ends at the termination of ventricular systole.

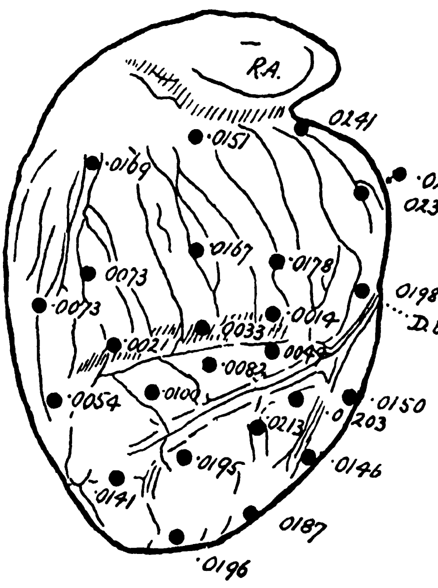

FIG. 356. Diagram showing the Time of Arrival of the Excitatory Wave at different points on the surface of the Ventricles in the Dog. (LEWIS and ROTHSCHILD, *Phil. Trans. Roy. Soc.*, 1915, **B206**, 181.)

Quite apart from the objections outlined above, we should not expect, in view of the mechanism of the propagation of the excitatory wave in the ventricle, that the cardiogram obtained in this indirect fashion would be

* CRAIB, *Heart*, 1927, **14**, 71; *J. Physiol.*, 1928, **66**, 49; GILSON and BISHOP, *Amer. J. Physiol.*, 1937, **118**, 743; WILSON, MACLEOD and BARKER, *J. Gen. Physiol.*, 1933, **16**, 423.

† LEWIS, MEAKINS and WHITE. *Phil. Trans. Roy. Soc.*, 1914, **B205**, 375.

easy of interpretation, at any rate so far as regards the course of the wave through the ventricular muscle. It must be remembered that the ventricular muscle is excited almost simultaneously at all points, in consequence of the rapid propagation of the wave of excitation along the branches of the A.V. bundle. The exact interval at which the excitatory process arrives at different points of the surface of the dog's ventricle after the upstroke of R in the whole electrocardiogram is shown, for example, in Fig. 356.

The exact form and time relations of the ventricular part of the electrocardiogram may be altered in disease, or by interruption of one or other of the main branches of the A.V. bundle, because then the order of spread of the wave of excitation becomes different.

DEXTRO- AND LÆVOCARDIOGRAMS. When the right branch of the A.V. bundle is blocked or cut, the excitatory wave will reach the left ventricle first, and if the left branch is divided, then the right ventricle will contract first. In either case the normal composite electrocardiogram will be departed from, and the resulting record is called lævocardiogram (*i.e.*, right branch blocked), or dextrocardiogram (left branch blocked) respectively, because it shows respectively the effect of an initial, and preponderating contraction of left or right ventricles. A similar record will be obtained if a premature contraction is provoked by a stimulus applied to the appropriate ventricle. The results of direct stimulation of the ventricles in man * show that the two curves have the following characters :—

Dextrocardiogram. *Lead I*, initial deflection (R) upwards ; *Lead III*, initial deflection (R) downwards.

Lævocardiogram. *Lead I*, initial deflection (S) downwards ; *Lead III*, initial deflection (R) upwards.

(Owing to incorrect inferences from other species, the earlier investigators believed that the deflections were the opposite of those stated above.)

The electrocardiogram, moreover, is of considerable use clinically for the determination of the relation between the auricular and the ventricular contractions. Thus, in heart block there will be P waves independently of Q, R, S waves ; in less developed form there will be delayed conduction time, as shown by increased P-R interval (over 0·2 sec.) ; in auricular fibrillation the P waves are replaced by small rapid waves, etc.

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FILLING OF THE HEART IN DIASTOLE. The heart during diastole exerts no active suction on the blood in the veins. Its filling depends on the existence of a pressure within the veins greater than that in the auricles and ventricles † ; the greater the pressure within the large veins the more rapidly will the blood enter the heart during diastole. In this process an important part is played by the mechanical conditions existing in the thoracic cavity. Owing to the elasticity of the lungs and to the fact that they are constantly tending to contract, the pressure in the thorax is less than that of the external atmosphere. At the end of expiration this difference amounts to about 5 mm. Hg, rising to 9 mm. Hg at the end of inspiration and to 30 mm. Hg at the end of a forced inspiration. This negative pressure chiefly affects the thin-walled veins and auricles, and the right ventricle when it is relaxed. On the other hand, the veins outside the thorax are

* BARKER, MACLEOD and ALEXANDER. *Amer. Heart J.*, 1930, **5**, 720.

† HENDERSON. *Amer. J. Physiol.*, 1906, **16**, 325.

exposed to a pressure which is a little above that of the atmosphere. The respiratory movements, by causing an alternating suction on the walls of the great veins and heart, act like an accessory pump and cause an aspiration of blood into the heart with each inspiration.

If the pressure within the thorax be raised enough to cause a sufficient positive pressure on the big veins and auricles, the return flow of blood into the heart is suspended. Thus during extreme muscular effort with the glottis fixed a positive pressure is produced in the thorax. The impaired circulation is rendered evident by the engorgement of the superficial veins. Weber showed that, by a forcible expiration with the glottis closed, the pulse might disappear at the wrist and the circulation be brought to a standstill for such a time that loss of consciousness might supervene.

The normal filling of the heart during diastole can be prevented by anything which hinders its expansion, such as the presence of fluid in the pericardial cavity. This happens in puncture of the left ventricle, as by rapier wounds. The same effect may be produced if oil be injected into the pericardium in a dog with the chest open ; when the pressure of the oil in the pericardial cavity is equal to that in the veins to the heart, the arterial blood pressure falls to zero and death ensues.*

It is under such conditions that the *pulsus paradoxus* may be observed, in which the pulse becomes small or absent during inspiration. This is due to the fact that, when the pericardial sac is filled with fluid under a positive pressure, the auricles and ventricles are much less affected by respiratory variations of intrathoracic pressure. The big veins, however, are still subject to these variations, so that during inspiration the blood tends to accumulate within them and not pass on into the heart. This organ therefore has less blood to discharge, and there is a diminution or absence of the pulse during inspiration. (Katz and Gauchat.)

The whole heart is enclosed in the tough, fibrous, inextensible sac formed by the pericardium. Under normal circumstances the heart, even during diastole, does not entirely fill the sac, increased rate of filling being compensated by a reflex quickening of the heart rate. Under extreme stress, when both inflow and resistance to outflow are increased, or when the heart muscle is in a bad condition from fatigue or disease, the heart may expand during diastole so as entirely to fill the pericardium, which then prevents any further distension, and, by limiting inflow, limits also the systolic output of the heart. Thus, the size of the pericardium determines the maximum possible output per beat. If the pericardium be removed, the heart may dilate in diastole still further, and the only limit to its filling is set by the elastic resistance of the heart wall. Under these conditions the forcible contraction of the heart walls may rupture the muscle fibres and lead to the ultimate failure of the heart.

SYSTOLIC OUTPUT OF THE HEART

The rate at which the blood circulates round the body depends on the quantity of blood which leaves the left heart per unit of time. The determination of the output of the left ventricle is therefore important. The output of the right ventricle must over any length of time be equal to that from the left ventricle, otherwise the blood would accumulate on one or other side of the heart. It must be remembered, however, that the coronary arteries, supplying the wall of the heart itself, leave the aorta at its very commencement, just beyond the aortic valves. The quantity of blood passing per

* KUNO. *J. Physiol.*, 1917, **51**, 221.

unit of time along the aortic arch is therefore less than that leaving the left ventricle by the amount of blood which traverses the coronary vessels. The true output of each ventricle per minute is therefore given, not by the flow through the aorta, but by the flow through the pulmonary artery (Anrep).

Attempts have been made to calculate the output of the heart from the pulse pressure and pulse wave velocity, but are highly theoretical. Methods for experimentally determining the cardiac output fall into two classes. In the first it is sought to determine the volume changes in the ventricles at each beat of the heart. A second method consists in the determination of the total volume of blood leaving the right or left ventricle in the course of a given time, say one minute. If this amount be divided by the number of heart beats in the same time, the output of each ventricle per beat (stroke volume) is obtained.

METHODS OF DETERMINING OUTPUT

(i.) Cardiometric Method. For recording the volume change of the heart per beat the cardiometer is used.* The chest and pericardium being opened, a glass cardiometer, of the shape shown in Fig. 257, is slipped over the heart. This cardiometer consists of a glass sphere with a wide opening. To the margin of the opening is tied a rubber diagram with a hole in it, which accurately fits the heart and lies in the auriculo-ventricular groove. The tube of the cardiometer is connected with some form of volume recorder, usually a piston recorder. During diastole the ventricles increase in volume; during systole they expel blood, and therefore diminish in volume. The change in volume at each beat gives the combined output of right and left ventricles, and must be divided by two in order to give the output of either ventricle separately. A cardiometer record is shown in Fig. 362. The disadvantage of this method is that the graphic record of rapid and ample changes in volume is difficult, the inertia and friction of the moving piston tending to deform the shape of the curve obtained.

(ii.) Application of the Fick Principle. If we know the difference between the oxygen content of the venous and arterial blood, it is clear that a determination of the oxygen intake by the lungs over a given interval of time will tell us how much blood has passed through the lungs during that time. This method has often been applied to intact animals, mixed venous blood being drawn from the right ventricle.

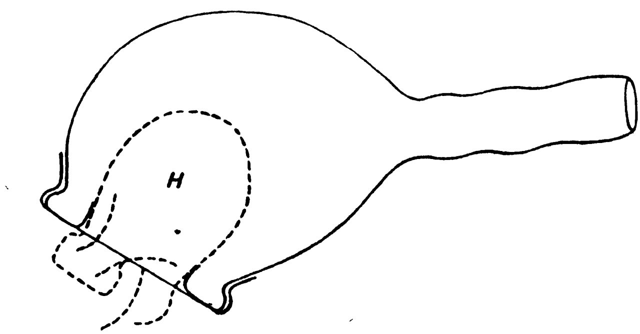

FIG. 357. Henderson's Cardiometer.

Thus† in one case, in a horse weighing 360 kilos, 2733 c.c. of oxygen were taken up in the lungs per minute, while the arterial blood contained 10·33 per cent. more oxygen than the venous blood. Since every 100 c.c. of blood that passed through the lungs had

* HENDERSON. *Amer. J. Physiol.*, 1906, 16 325.

† ZUNTZ and HAGEMANN. "Stoffwechsel des Pferdes," 1898, p. 372. (Berlin, Parey.)

taken up 10·33 c.c. of oxygen, and 2733 c.c. had been taken up in the course of a minute, it follows that

$$\frac{100 \times 2733}{10 \cdot 33} = 26,457 \text{ c.c.}$$

of blood must have passed through the lungs in the time. This was the output of blood by the right ventricle in a minute and was equivalent to 0·00122 of the body weight per second. Instead of oxygen differences, CO_2 differences may be taken, and the total output of CO_2 by the lungs determined.

Determination of Cardiac Output in Man. One of the methods for determining the minute output or *minute volume* of the heart in man is similar to the method described above, in that the volume of blood passing through the lungs is calculated from the output of CO_2 by the breath during a given time compared with the amounts of this gas in arterial and venous bloods respectively. Indirect methods are used to find the volume of CO_2 in the arterial and venous bloods of man without the use of operative procedures. In Field's modification of Barcroft's and Haldane's methods, the tension of CO_2 in the arterial blood is obtained by determining the amount of this gas in ordinary alveolar air, since the alveolar tension of CO_2 is identical with the tension of this gas in the arterial blood. This tension must be converted into percentage content in blood by obtaining a sample of blood and finding the amount of CO_2 it will take up at that tension.

The content of CO_2 in the venous blood coming into the lungs is determined as follows :—The subject, having expired deeply, takes three deep breaths in succession of a mixture containing about 94 per cent. oxygen and 6 per cent. CO_2, *i.e.* just above what is known to be the tension of CO_2 in arterial blood. The large amount of oxygen is supplied in order to prevent errors due to anoxæmia and consequent alteration in the CO_2 dissociation curve of the blood. These three breaths are taken within fifteen seconds, and the last one is held for a few seconds. As the total circulation time in a man at rest is about thirty seconds, there is no time for the blood which has taken up CO_2 from the lungs to come back to the lungs, *i.e.* equilibrium is established between the air in the alveoli and the continually renewed venous blood arriving at the alveoli by the pulmonary artery. After holding the third breath the alveolar air is collected. This is in equilibrium with the pulmonary venous blood, and the tension of CO_2 in the latter can be calculated from the amount of CO_2 in the alveolar air.* This is converted into percentage of CO_2 in the venous blood by reference to the CO_2 dissociation curve, as was done for arterial blood. In this way we find the difference between the CO_2 content of arterial and venous blood, *i.e.* of the blood leaving and of the blood entering the lungs. Before these determinations are carried out, the total CO_2 given off by the individual is measured by collecting his expired air for ten minutes in a Douglas bag. Suppose that the difference in CO_2 content of arterial and venous blood was 40 c.c. per litre blood, while the total amount of CO_2 given off from the lungs in the course of a minute was 200 c.c., the total amount of blood would be $\frac{200}{40} = 5$ litres, which would thus be the 'minute' volume of the heart of the subject in question.

In other methods the arterio-venous oxygen difference is determined.

Krogh and Lindhard † devised a method for determining the blood flow through the lungs by finding how much nitrous oxide is taken from a mixture of nitrous oxide and air with which the lungs are filled. Knowing the solubility coefficient of nitrous oxide in blood at the tension and temperature at which this gas is present in the pulmonary alveoli, and determining the amount of this gas which has disappeared from the mixture breathed during a certain time, it is possible to estimate the volume of blood which has passed through the lungs during the same time.

Similar methods, employing other gases, have been used. One of the simplest and best is acetylene, of which at 760 mm. 1 l. of blood dissolves 740 c.c. : it is employed by Marshall and Grollman ‡ as follows: The oxygen consumption per

* There are differences of opinion about the justifiability of the assumption that equilibrium is reached, cf. Israëls and Lamb, *J. Physiol.*, 1929, **67**, 315 ; Hamilton *et al.*, *J. Physiol.*, 1930, **70**, 244.

† Krogh and Lindhard. *Skand. Arch. f. Physiol.*, 1912, **27**, 100 ; Krogh, *ibid.*, p. 227 ; Lindhard, *J. Physiol.*, 1923, **57**, 17.

‡ Marshall and Grollman. *Amer. J. Physiol.*, 1928, **86**, 117 ; Grollman, *ibid.*, 1929, **88**, 432.

minute of the subject is first determined. He then re-breathes, from a rubber bag of 3 l. capacity, a mixture of about 10 per cent. acetylene, 20 per cent. oxygen, and 70 per cent. nitrogen, for a few seconds to attain equilibrium of the mixture in the lungs plus bag. A sample of the gas is collected and, after a further 5 seconds' re-breathing, a second sample. The two samples are analysed, and the diminution of oxygen and of acetylene in the second sample (due to their being carried away by the blood circulating through the lungs) is found. The arterio-venous oxygen difference, in cubic centimetres of oxygen per litre of blood passing the lungs $= \frac{O_2\text{Diff.}}{C_2H_2\text{ Diff.}} \times 740 \times \frac{B - 48\cdot 1}{760} \times \frac{(C_2H_2)\text{ average}}{100}$, where "$C_2H_2$ average" is the mean concentration of acetylene, B the barometric pressure and 48·1 is the tension of aqueous vapour at body temperature. The output per minute of the heart in litres

$$= \frac{\text{Oxygen consumption c.c. per minute}}{\text{Arterio-venous oxygen difference}}.$$

In normal fasting men at rest the output is about 3 to 4 l. per minute, or $2\cdot 2 \pm 0\ 3$ l. per minute per sq. metre of body surface, this figure being called the *cardiac index*. It falls slightly with advancing age, from forty to fifty-five, and then more slowly.*

THE BALLISTOCARDIOGRAM. When the heart suddenly expels its blood into the arteries during systole, a recoil (comparable with that of a rifle, together, sometimes, with the rifleman, when a bullet is fired) is given to the heart and to the whole body. If one stands on a delicate spring weighing-machine, the pulsation due to this ballistic recoil, is clearly shown as a pulsation. A curve, the ballistocardiogram can be recorded by having the subject lie on a suspended support movable in the long axis of the body.† It shows important changes with altered heart output.

CARDIO-PNEUMATIC MOVEMENTS. Since the heart during its systole diminishes its own volume by the expulsion of blood from the thorax, it becomes smaller, and the space thus provided in the chest cavity is taken up by an expansion of the veins, auricles, and lungs. To this systolic diminution of intrathoracic pressure are due the 'cardio-pneumatic' movements. These are recorded by attaching one nostril to a delicate tambour by means of a tube, while the other nostril and the mouth are kept closed. If a carotid pulse tracing be taken at the same time, it will be found that there is a fall of the pressure in the nasal cavity, synchronous with the rise of the pressure in the arteries, both being due to the expulsion of blood from the heart.

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* LEWIS. *Amer. J. Physiol.*, 1938, **121**, 517.
† STARR and RAWSON. *Amer. J. Physiol.*, 1938, **123**, 195.

CHAPTER XXXII

FACTORS WHICH MODIFY THE HEART'S ACTION

THE performance of the heart is subject to considerable alterations, due to the operation of numerous factors capable of affecting either the frequency or the extent of its contractions. With the heart *in situ* these factors are difficult to analyse, owing to their number and complex interactions, particularly those of the mutual effects of the systemic circulation and the heart beat upon one another. In order to be able to study the conditions which can control the filling and emptying, and also the rate, of the heart in the absence of the complications normally introduced by the presence of a delicately poised and constantly changing peripheral circulation, it is of importance to be able to replace the latter by an artificial circulation, the resistance of which can be controlled at will. This can be effected by the use of the heart-lung preparation.

The Heart-Lung Preparation. With the use of this preparation, devised by Starling,* it is possible to study the action of the mammalian heart under all manner of

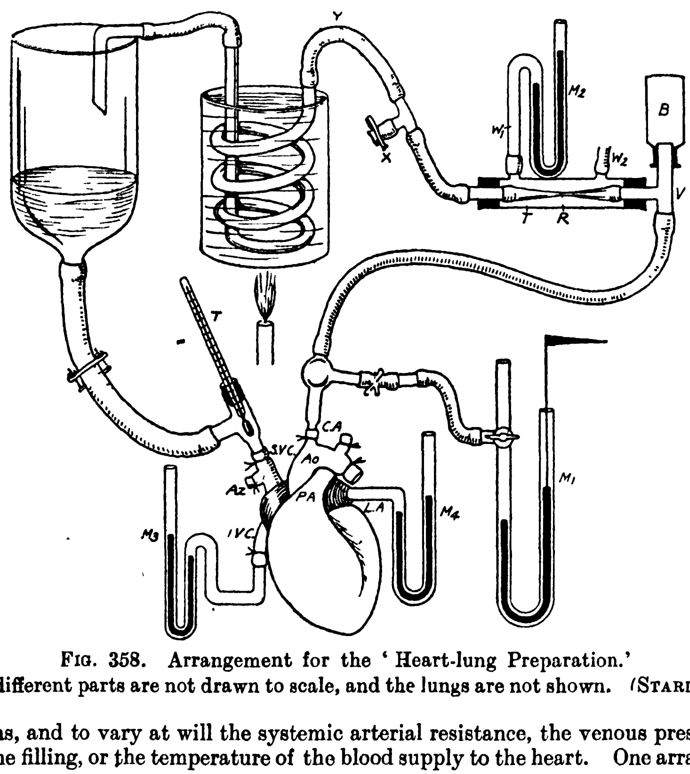

FIG. 358. Arrangement for the 'Heart-lung Preparation.'
The different parts are not drawn to scale, and the lungs are not shown. (STARLING.)

conditions, and to vary at will the systemic arterial resistance, the venous pressure, the rate of the filling, or the temperature of the blood supply to the heart. One arrangement

* JERUSALEM and STARLING. *J. Physiol.*, 1910, **40**, 285, modified by KNOWLTON and STARLING, *ibid.*, 1912, **44**, 206; and PATTERSON and STARLING, *ibid.*, 1914, **48**, 357. A closed circuit system has been devised by DALY, *J. Physiol.*, 1925, **60**, 103.

of the apparatus is shown in Fig. 358 Artificial respiration being maintained, the chest is opened under an anæsthetic. The arteries coming from the arch of the aorta—in the dog, the brachio-cephalic and the left subclavian—are then ligatured, thus cutting off the whole blood supply to the brain, so that the anæsthetic can be discontinued. Cannulæ are placed in the brachio-cephalic artery and the superior vena cava. The cannulæ and the large venous reservoir are filled beforehand with defibrinated blood. The

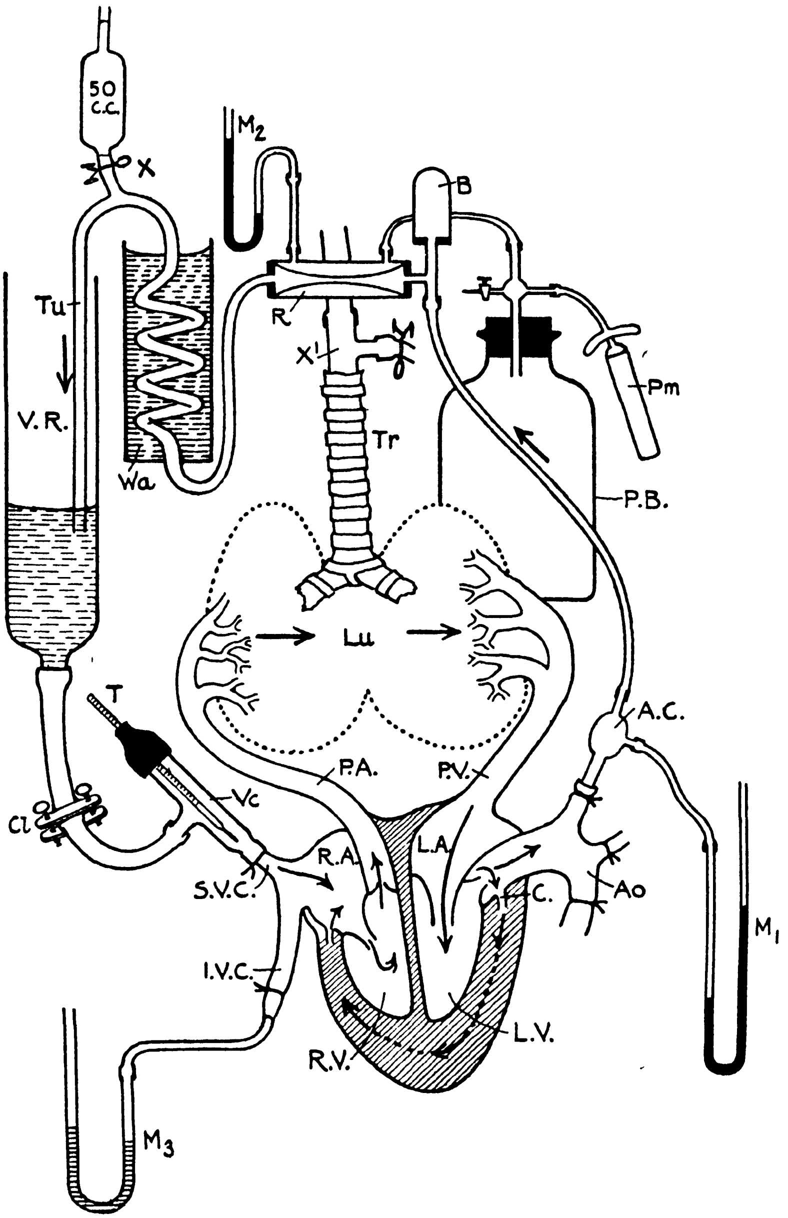

FIG. 359. Diagram of Circulation in Heart-Lung Preparation. P.B., Pressure bottle. Pm, Pump. Wa, Warming apparatus. V.R., Venous reservoir. Cl, Adjustable clamp. A.C., Arterial cannula. R.V. Right, and L.V., Left ventricle. C., Coronary supply. P.V., Pulmonary vein. Lu, Lungs. Tr, Trachea. X^1, Tracheal tube. Other letters as in Fig. 358. Direction of blood flow indicated by arrows.

descending aorta is closed by a ligature. The only path left for the blood is by the ascending aorta and the cannula *CA* in the brachio-cephalic artery. The arterial cannula communicates by a T-tube with a mercurial manometer M^1 to record the mean arterial pressure, and passes to another T-tube *V*, one limb of which projects into a bottle *B*. The air in this vessel will be compressed with a rise of pressure and will serve as a driving force for the blood through the resistance. It thus takes the part of the resilient arterial wall. The other limb of the T-tube passes to the resistance *R*. This consists of a thin-walled rubber tube (*e.g.* a rubber fingerstall) which passes through a wide glass tube provided with two lateral tubulures W_1, W_2. One of these is connected with a mercurial manometer M^2 and the other, W_2, with an air reservoir into which air can be pumped. When air is injected into the outer tube, the tube *R* collapses, and will remain collapsed until the pressure of the blood within it is equal or superior to the pressure in the air surrounding it. It is thus possible to vary at will the resistance to the outflow of the blood from the arterial side. From the peripheral end of *R* the blood passes at a low pressure through a spiral immersed in warm water into a large glass reservoir. From the reservoir a wide india-rubber tube leads to the cannula, which is placed in the superior vena cava *SVC*, all the branches of which have been tied. This cannula is provided with a thermometer to show the temperature of the blood supplied to the heart. A tube placed in the inferior vena cava and connected with a water manometer M_2 shows the pressure in the right auricle. On the recording surface we thus have a record of the arterial pressure, and of the pressure within the right auricle. The rate of inflow from the reservoir into the right auricle is controlled by an adjustable screw-clamp. The output of the whole system can be measured at any time by opening the tube *X*, clamping *Y*, and allowing the blood to flow for a given number of seconds into a measure. The course of the circulation is shown in Fig. 359.

This method, although of considerable value in giving information as to the conditions which determine the output of the left ventricle and the maximum capacity of the heart as a pump, tells us nothing as to the output of the left ventricle under normal conditions in the intact animal. Moreover the output as measured represents the ventricular output minus the blood flow through the coronary arteries. It is possible, however, to insert a cannula into the coronary sinus, and so to measure the blood flow through the heart muscle.* The coronary circulation must be added to the flow through the arterial resistance in order to arrive at the correct total output of the left ventricle. An essential in this preparation is that the output from the left ventricle and the pressure in the aorta must be sufficient to maintain a circulation through the coronary vessels.

OUTPUT AND INFLOW. It is self-evident that provided the venous inflow remains constant, the minute output, when considered over a reasonable interval of time, must also be constant, equal to the inflow, and unaffected by considerable alterations of arterial resistance or heart rate. Thus, in the heart-lung preparation with a moderate and constant venous inflow the output remains constant whether we maintain the average arterial pressure at 60 mm. Hg or at 160 mm. Hg. It is also unaffected by altering the rate of the heart from 80 beats per minute up to 160 or even 200 beats per minute. On the other hand, the output must at once be altered by changes in the venous inflow and can in fact be varied in a dog's heart weighing 50 gms. from 100 c.c. up to 3000 c.c. per minute.

With increasing inflow of blood into the heart, the large veins, auricles and ventricles become more filled during diastole; and during systole of the ventricles, when the auriculo-ventricular valves are closed, the incoming blood must accumulate in the big veins and auricles to a still greater extent. The venous pressure in the intact animal therefore rises, with increased venous inflow. In so far as venous pressure is an index of venous inflow, we may say that the output of the heart increases with the venous pressure, so long as the heart is functionally capable of dealing with the blood it receives during diastole. And since the ventricular output in the isolated heart is practically independent of the frequency

* Evans and Starling. *J. Physiol.*, 1913, 46, 413.

of the beat, it follows that with a constant venous inflow the venous pressure tends to fall, and diastolic volume to diminish, as the heart rate increases. The optimum venous pressure is that which fills the ventricle during its diastole to the maximum extent to which it is able to respond. As the rate of the heart increases, the inflow of blood can also be increased without causing over-distension of the ventricles. An increase of heart rate is therefore, in the intact animal, an important factor in enabling this organ to deal with the maximum amount of blood. Although increase of rate does not alter the output with constant venous inflow, it does increase the *maximum* amount of inflowing blood which the heart is able to expel. At the same time, the lowering of pressure in the great veins near the heart increases the fall of pressure from the extrathoracic to the intrathoracic veins, and so aids the venous inflow into the chest.

As might be expected, the alterations in the vigour of the circulation depend in the first instance on the venous circulation. The greater the volume of the blood that is brought up to the heart by the accessory factors of the circulation, the greater will be the output of this organ. The changes in rate and force of the heart which accompany its increased activity and increased output, *e.g.* during exercise, represent merely the response by which this organ is able to deal in the most advantageous manner with the increased inflow.

The cardiac output, like the oxygen consumption of the individual, is definitely increased after ingestion of food (Fig. 360). It is also greater in

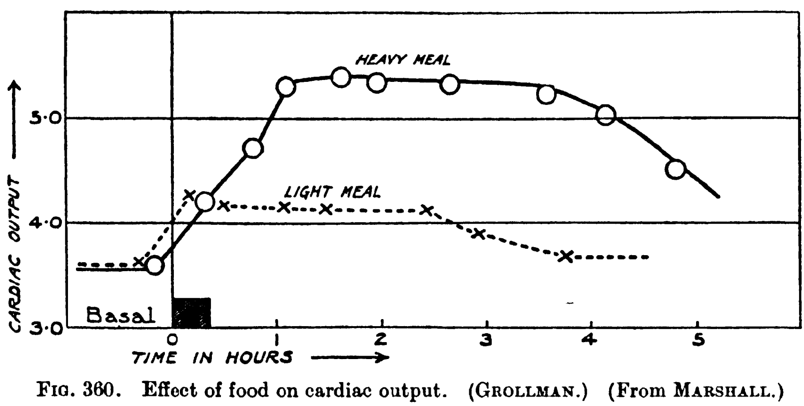

FIG. 360. Effect of food on cardiac output. (GROLLMAN.) (From MARSHALL.)

man by 10 or 12 per cent. when lying supine, than when sitting, and becomes still smaller when standing erect.*

THE WORK OF THE HEART. The energy of the ventricular contraction is expended in two ways: first, in forcing a certain amount of blood into the already distended aorta against the resistance presented by the arterial blood pressure; and secondly, in imparting a certain velocity to the mass of blood so thrown out. Thus the energy of the muscular contraction is converted partly into potential energy in the form of increased distension of the arterial wall and partly into the kinetic energy represented by the momentum of the moving column of blood. The work done at each beat may be calculated from the formula:

$$W = QR + \frac{wV^2}{2g}$$

* DONAL, GAMBLE and SHAW. *Amer. J. Physiol.*, 1934, **109**, 666; MCMICHAEL. *Q. J. Exp. Physiol.*, 1937, **27**, 55.

where W stands for work, w for the weight, and Q for the quantity (volume in c.c.) of blood expelled at each contraction; R is the average arterial resistance or pressure in terms of height of a column of blood, during the outflow of blood from the heart, and V is the mean velocity at which the blood is expelled. In this equation QR is the work done in overcoming the resistance, and $\frac{wV^2}{2g}$ is the energy expended in imparting velocity to the blood.

If we take 60 c.c. as the average output of each ventricle, 100 mm. Hg as the average pressure at the beginning of the aorta, and 500 mm. per second as the velocity imparted to the blood thrown into the aorta, we can roughly calculate the work done by the human heart at each beat:—

$$QR = 60 \times 0{\cdot}100 \text{ m.} \times 13{\cdot}6 = 81{\cdot}6 \text{ gram-metres},$$

or roughly 80 gram-metres. On the other hand, the expression

$$\frac{wV^2}{2g} = \frac{60 \times (0{\cdot}5)^2}{2 \times 9\,8} = 0{\cdot}7 \text{ gram-metres.}$$

It is evident that this latter factor is negligible, and that with small outputs we may regard the work of the heart as sufficiently expressed by the output multiplied by the average arterial blood pressure. Taking the average pressure in the pulmonary artery at 20 mm. Hg, the work of the right ventricle at each beat would amount to about 16 gram-metres, a total for the two ventricles of about 100 gram-metres per beat, which is equivalent to about 10,000 kilogram-metres in twenty-four hours for a man at rest.

During muscular work this figure would be largely increased. Not only does QR become much larger, but the velocity factor is no longer negligible, since the work done in imparting velocity to the blood increases as the cube of the output per minute. If we take, as an example, a maximum effort on the part of an athlete, we may assume an output per beat of 180 c.c. and a pulse rate of 180 per minute (an output per minute of 32·4 litres) and an average arterial pressure of 120 mm. Hg.

Then

$$QR = 180 \times 0{\cdot}120 \times 13{\cdot}6 = 294 \text{ gram-metres.}$$

To determine the velocity of output, we assume that 180 c.c. of blood are thrown out into the aorta during $\frac{1}{8}$ second. This gives a velocity of 2·3 metres per second, assuming a cross section of 625 mm.^{2} at the root of the aorta. Therefore

$$\frac{wV^2}{2g} = \frac{180 \times (2{\cdot}3)^2}{2 \times 9{\cdot}8} = 49 \text{ gram-metres.}$$

The total work of both sides of the heart will be:

$$\underbrace{294 + 49}_{\text{Left side.}} + \underbrace{59 + 49}_{\text{Right side.}} = 451 \text{ gram-metres per beat, or 81 kilogram-metres per minute.}$$

It will be noted that now the velocity-factor accounts for nearly one-quarter of the total work. This rate of work could probably not be maintained for more than a few minutes.

Since the period of systolic output occupies not more than $\frac{3}{8}$ of the cardiac cycle, the mean velocity during expulsion must be $2\frac{2}{3}$ times greater than the mean aortic velocity: the following general formula $W = \frac{7QR}{6} + \frac{w(VC)^2}{gE^2}$, (where V is the mean aortic velocity, C the duration of the cardiac cycle and E the duration of period of expulsion) gives the work of both ventricles.* The expression QR is also only

* LOVATT EVANS. *J. Physiol.*, 1918. 52. 6.

approximately correct. Supposing the pressure in the aorta at the beginning of systole is 50 mm. Hg, and at the end of systole 150 mm., the work could not be deduced accurately from the average pressure, but would need a simple application of the integral calculus for its determination. This has been made by Katz,* who finds that the expression employed above deviates from the real value by a variable amount, and tends to underestimate the work done, especially that due to the kinetic factor.

It is important to remember that the strain or tension, which is thrown on the cardiac fibres and which resists their contraction, will be determined not only by the blood pressure which has to be overcome, but also by the size of the ventricle cavities. If we regard the ventricle as a sphere, it is evident that, in order to produce a pressure p per unit area, in its interior, the total pressure exerted by the walls must be $4\pi r^2 p$, *i.e.* it must be proportional to the surface area of the inside of the ventricle or to the square of its radius. Moreover in the case of the heart, with increasing distension, the wall becomes thinner and the number of muscle fibres in a given area fewer, so that the larger the heart the more strongly will each fibre have to contract in order to produce a given tension in the contained fluid. At the beginning of systole the distended heart must therefore contract more strongly than at the end of the systole, if it is to develop an endocardiac pressure sufficient to overcome that in the aorta.

ADJUSTMENT OF OUTPUT TO INFLOW. When we examine the behaviour of a heart isolated from the rest of the body, as in the heart-lung preparation, we find that it has a marvellous power of adaptation, *i.e.* of regulating its activity according to the mechanical demands which are made upon it. Thus, if we keep the venous inflow constant, it makes no difference to the output of the heart whether the average arterial pressure be maintained at 80 or 160 mm. Hg, although in the latter case the heart must do twice as much work in order to keep the outflow at the same level. We could compare this behaviour with that of an automobile with a marvellous automatic throttle so designed as to give a constant velocity, whatever the load or the gradient, throughout the journey. Again, if we maintain the arterial pressure constant and alter the venous inflow, we find that within very wide limits the heart is able to expel against the arterial resistance the whole of the blood which flows into it from the veins. In this way we can alter the output of a small heart of 50 grammes from 300 to 3000 c.c. per minute. To use our automobile analogy again, the throttle is designed to enable it to perform any reasonable journey, long or short, in the same time.

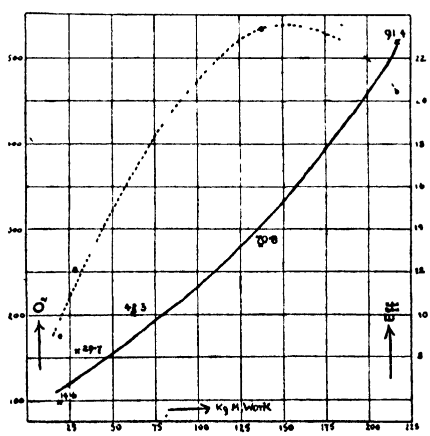

FIG. 361. Oxygen Usage and Efficiency of Dog's Heart (H–L–P), showing their Relation to Work done. Arterial pressure, 80 mm. Hg throughout. Continuous line, oxygen usage, c.c. p. hr.: the figures on the oxygen curve show the output in l. p. hr. Dotted line, efficiency. (LOVATT EVANS, 1918.)

Oxygen Usage and Efficiency. As we should expect, this variation in the work done by the heart is associated with corresponding variations in the chemical changes which occur at each heart beat. It is easy with the heart-lung-preparation to determine the actual work done and to vary this at will. Then, knowing that each cubic centimetre of oxygen used represents the liberation of about 2 kg.-m. of energy, it is easy to calculate at what mechanical efficiency the heart is working over any given period of

* KATZ. *Amer. J. Physiol.*, 1932, 99, 579.

observation. Thus, if a heart uses 3,000 c.c. of oxygen in a given period it has liberated as heat and work about 6,000 kg.-m. of energy; if in that time it performed 600 kg.-m. of work the efficiency was 0·1 (or 10 per cent.). In this way it was found that the efficiency of the heart is probably never greater than 30 per cent.—more usually it is from 5 to 10 per cent. In this respect the heart shows much the same mechanical performance as the skeletal musculature. The respiratory exchange of the heart increases *pari passu* with the work it has to do.* Thus in an isolated dog's heart, weighing 70 grammes, with a constant output of 35 litres per hour, raising the arterial pressure from 80 mm. Hg to 140 mm. Hg increased the oxygen consumption from 228 to 404 c.c. per hour. In another experiment with a heart of 59 grammes, in which the arterial pressure was maintained constant at 80 mm. Hg, increasing the output from 9·3 to 92 litres per hour raised the oxygen consumption from 155 to 649 c.c. per hour. When the efficiency of the heart at various levels of work is plotted against the mechanical work done, it is seen to reach a maximum with increasing work, and then to decline (Fig. 361). Increase of the work beyond this point soon leads to insufficiency: over a certain further range more work can be accomplished but at rapidly increasing cost, and with still further increase of resistance or inflow the mechanical work done also declines and failure is then imminent.

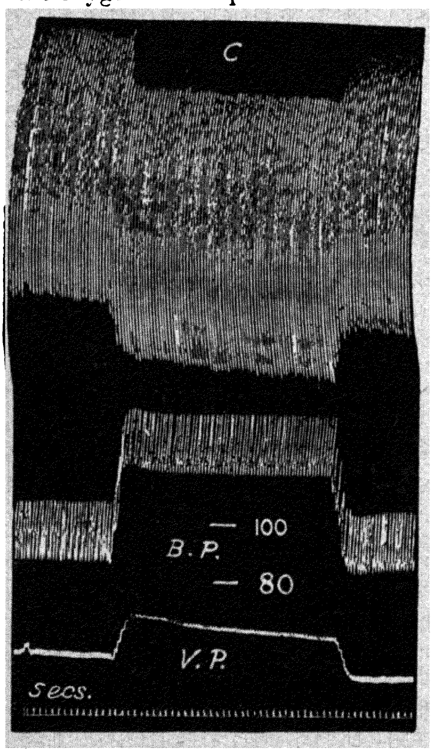

FIG. 362 Effect of Increased Arterial Pressure on the Volume Changes of the Heart, with a steady inflow of 154 c c. Blood per 10 seconds.

C. = cardiometer curve. B.P. = arterial blood pressure. V.P. = pressure in the inferior vena cava. The lines 100 and 80 are calibrations for the blood pressure in mm. Hg. (PATTERSON, PIPER and STARLING.)‡

The Law of the Heart. Investigation of the volume and pressure changes of the heart under varying conditions of arterial resistance and venous filling throws light on the mechanism of this adaptation.† Let us take first the changes in volume as recorded by the cardiometer, and suppose a heart is contracting 100 times per minute, forcing out at each beat 10 c.c. of blood into the aorta against an average pressure of 100 mm. Hg, with systolic and diastolic pressures respectively of 110 and 90 mm. Hg (Fig. 362). In order that the left ventricle may force 10 c.c. of blood against this resistance, the pressure in its interior must rise at each heart beat above the maximum systolic pressure in the aorta, *e.g.* to 120 mm. Hg. The aortic valves will open as

* EVANS and MATSUOKA. *J. Physiol.*, 1914, **49**, 378.
† PATTERSON and STARLING. *J. Physiol.*, 1914, **48**, 357; PATTERSON, PIPER and STARLING, *ibid.*, p. 465.
‡ PATTERSON, PIPER and STARLING. *J. Physiol.*, 1914, **48**, 465.

soon as the pressure rises above 90 mm. Hg. Suppose the arterial resistance is now increased so as to bring the average pressure up to 130 mm. Hg. The heart now may raise the pressure in its interior to 130 mm. Hg. This will be higher than the diastolic pressure in the aorta and a certain amount of blood will escape, but the outflow of blood will cease as soon as the pressure in the aorta is equal to that in the ventricle. Diastole will then occur, the ventricle will relax before it has emptied out 10 c.c. of blood. Let us assume it has forced out 3 c.c. of blood, so that the excess of 7 c.c. remains at the end of systole. Meanwhile, the venous inflow is proceeding at the same rate as before, so that at the end of diastole its diastolic volume will be increased by 7 c.c. At the next beat we find that the contraction of the ventricle is much more forcible. The maximum pressure now rises to 140 mm. Hg and 8 c.c. of blood are sent out into the aorta. At the end of this beat the heart will be still fuller than before, containing an excess of 9 c.c. of blood. The third beat is still more forcible, the intraventricular pressure rising to a maximum of 145 mm. Hg, and now 10 c.c. of blood are

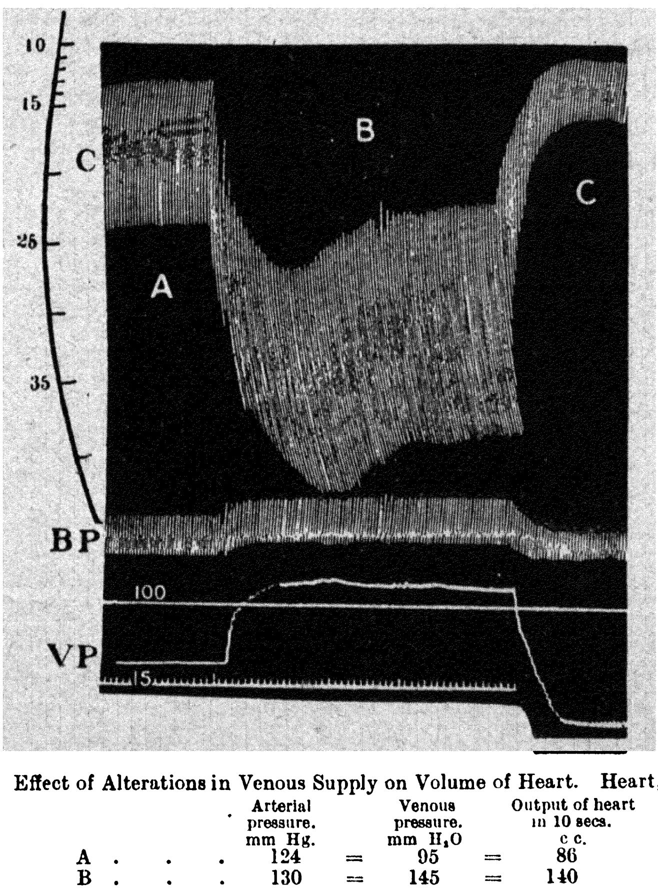

FIG. 363. Effect of Alterations in Venous Supply on Volume of Heart. Heart, 67 grms.

	Arterial pressure. mm Hg.		Venous pressure. mm H_2O		Output of heart in 10 secs. c.c.
A . . .	124	=	95	=	86
B . . .	130	=	145	=	140
C . . .	124	=	55	=	33

The curved line at the side represents the value of the cardiometer excursions, *i.e.* of alterations of ventricular volumes, in cubic centimetres. (PATTERSON, PIPER and STARLING.)*

* *Loc. cit.*

expelled. After this, the heart goes on beating regularly, expelling 10 c.c. of blood at each beat, *i.e.* the same amount as it receives from the veins. But the heart remains more dilated than it was previously (Fig. 362), since it contains an excess of 9 c.c. of blood. If now the arterial resistance be suddenly reduced to its previous amount, the first beat after the change may send out 17 c.c. of blood, the second beat 12 c.c. of blood and the third beat 10 c.c. as before. It is similar with alterations of the rate of inflow, with constant arterial pressure, as shown in Fig. 363. We see, therefore, that an increased liberation of energy results from the contraction of the heart muscle when there is an increased diastolic volume of the heart and *vice versâ*; but increased diastolic volume means greater length of the muscular fibres composing its wall, so we arrive at a statement that, as in voluntary muscles, the *energy of contraction is a function of the length of the muscle fibres*. This important generalisation is commonly called Starling's 'Law of the Heart.' *

This reaction of the heart to increasing distension has long been known, but was ascribed to the excitatory influence of tension on the muscle fibres. In a resting heart increasing distension of its cavities will tend to stretch its muscle fibres and therefore to exert a tension on them. By accurately recording the pressure changes within the contracting ventricle under varying conditions, it has been found that the tension on the fibres is not the determining factor. In a heart beating regularly, the inflow of blood is proceeding during the relaxation of the ventricles, *i.e.* the muscles are giving before the inflowing blood. The latter is therefore able to distend the heart without exercising more than a minimum pressure on its walls. With a small inflow it is found that the pressure in the ventricles may be approximately zero at the end of diastole, whether the heart is contracting against a resistance of 80 mm. Hg or a resistance of 120 mm. Hg, or, with a moderate arterial resistance, whether it is receiving 5 c.c. or 10 c.c. during the period of diastole. With a still larger outflow or a bigger resistance there is an appreciable

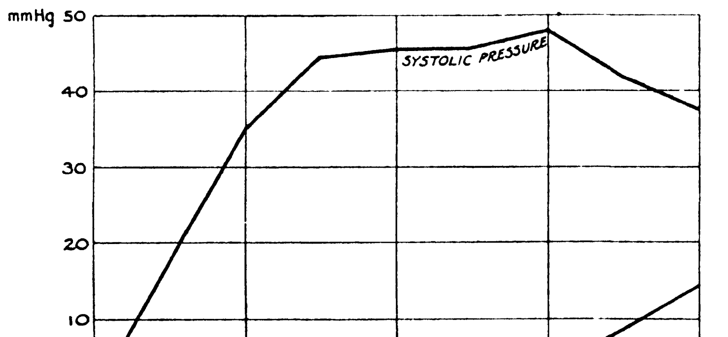

FIG. 364. Relation of the Tension developed during Isometric Contraction of the Tortoise Ventricle to the Filling (*i.e.* initial length of fibres) of the Ventricle. (KOZAWA.)†

rise of tension within the ventricles at the end of diastole. But that the change in *distension, i.e.* in the diastolic length of the muscle fibres, is the chief factor in determining the energy of the subsequent contraction is evident on reference to Fig. 364, which shows the reaction to increasing filling of the tortoise ventricle contracting isometrically. It will be seen that as the diastolic filling increased from 0 to 1·5 c.c., the intraventricular pressure developed during systole increased from 0 to 45 mm. Hg. The maximum systolic pressure (48 mm. Hg) was developed with a filling of 3 c.c. and an initial tension

* STARLING. Linacre Lecture, "The Law of the Heart." (London, 1918.)
† KOZAWA. *J. Physiol.*, 1915, **49**, 233.

within the heart of 3 mm. Hg. Further increasing the filling to 4 c.c. caused for the first time a considerable rise of initial tension to 13 mm. Hg, but the result was to *diminish* the tension developed during systole. This means that, up to a certain optimum length, the energy of contraction increases with every increase in initial length of the fibres. Beyond this point it requires considerable tension to cause a further increase in length, but this is associated with a diminution in the effectiveness of the contractions.

The capacity of the heart for adapting itself to changes in mechanical demands made upon it will be limited by the inability of the heart to dilate further, as is probably the case in the intact animal, where its dilatation is limited by the pericardium, and by the mechanical disadvantage at which the further dilated heart acts. The greater its diastolic volume, and the more nearly globular its form, the greater the mechanical disadvantage of the muscle fibres in raising the pressure in the interior of the ventricles, so that by continually increasing the demands on the heart we shall finally arrive at a stage at which this organ is unable to deal with the blood supplied to it and rapidly fails to expel any of its contents.

Evidence of a relation between heart volume and total energy consumption was furnished by the demonstration, by Evans and Matsuoka, that there is a parallel between heart volume and oxygen usage. Subsequently Anrep showed that in the isolated heart, within physiological limits, a given increase in the total *work* of the ventricle, whether brought about by augmented inflow or by a rise in the arterial resistance, is always accompanied by the same increase in the diastolic volume of the ventricles. Determinations of the oxygen usage of the heart * also show that, within limits, when the diastolic volume is the same, the oxygen usage is the same, whatever may be the amount of work done (Fig. 365): in other words, the oxygen utilisation, the work done, and the diastolic volume are closely correlated, the first two being probably determined by the last, over rather wide ranges.† Beyond the point of maximal performance this relation no doubt breaks down, diastolic volume and oxygen utilisation showing a further increase, but the work accomplished diminishing; this holds good in general whenever, with an increased volume in diastole, there is a reduced efficiency, as is the case when the heart fails. This was confirmed by Stella,‡ who used the tortoise heart and found that the relationship between diastolic volume and oxygen usage depends upon the conditions under which the heart works.

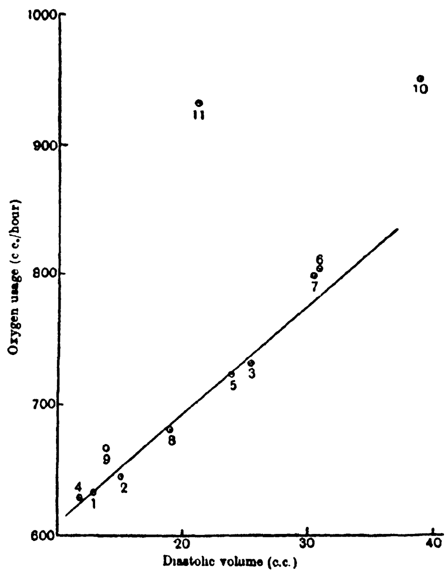

FIG. 365. Showing Relation between Heart Diastolic Volume and Oxygen Usage = Abscissæ alteration in diastolic volume, in c.c. Ordinates = oxygen usages in c.c./hr. for whole heart-lung preparation. (HEMINGWAY and FEE. *J. Physiol.*, 1927, **63**, 299.)

The physiological condition of the heart is measured by the maximum pressure which it is able to produce in its cavities when it contracts, starting from a certain initial size or length of fibres. As the heart becomes fatigued this pressure falls, so that the heart must dilate in order that each contraction shall produce the same maximum pressure as before. Dilatation is therefore a measure of fatigue.

* VISSCHER and STARLING. *J. Physiol.*, 1927, **62**, 243.
† HEMINGWAY and FEE. *J. Physiol.*, 1927, **63**, 299.
‡ STELLA. *J. Physiol.*, 1931, **72**, 247.

THE HYPODYNAMIC OR INSUFFICIENT HEART is unable properly to cope with the work which it is set to do. Sooner or later all isolated hearts become insufficient, a condition which is revealed by a rising intra-auricular pressure. As the heart approaches insufficiency it tends to do less work, and to dilate. The dilatation is always accompanied by an increased oxygen usage. If now the work conditions are lightened the dilatation and oxygen consumption may be reduced, and the heart may be competent to carry on at the easier level. If, on the contrary, still further work is offered to an already insufficient heart the mechanical work done *may* be restored to its former level, but only at the cost of a still further dilatation, rise of intra-auricular pressure, and greatly augmented oxygen usage. Like all pathological phenomena, these effects are nothing more than exaggerations of normal properties, and as Starling and Visscher pointed out, insufficiency is mainly a reduced mechanical efficiency. They supposed that, of the expended chemical energy (as judged by oxygen usage) at a given diastolic volume, a smaller fraction was converted into mechanical work by the failing than by the normal heart.

Addition of digitalis or strophanthin to the blood leads to a real improvement of the insufficient heart, *i.e.*, at a given task the diastolic volume and oxygen consumption of the spontaneously insufficient heart are reduced and hence the efficiency is increased.

TONE. Many authors speak of a 'tone' of the heart muscle, generally meaning thereby a tendency to resist distension during diastole. This term is ambiguous, and has been used in so many meanings that it would be better to avoid the word altogether. The 'tone of the heart' is really synonymous with physiological condition. A heart in good condition has a 'high tone.' It empties itself almost completely at each beat, even when receiving a considerable quantity of blood during diastole. A heart with a 'low tone' is in the condition of a fatigued heart. It is greatly dilated and when it has finished contracting still contains a large amount of residual blood.

COMPENSATION. This property of the cardiac muscle is responsible for the power of 'compensation' possessed by a diseased heart. We may take as an example the destruction of one aortic valve, a lesion which can be produced experimentally in a dog. In this case, immediately after the lesion is established, no additional resistance is offered to the expulsion of the blood, and the ventricle will send the normal amount into the aorta. During the succeeding diastole the blood at a high pressure in the aorta will leak back into the ventricle through the damaged valve. The arterial pressure therefore falls rapidly, and the ventricle receives blood from two. sides, *i.e.* by regurgitation through the aortic valves, and in the normal way from the auricles and veins. At the end of diastole the ventricle is therefore overfilled. Increased stretching of its fibres, however, has the effect of producing an increased contraction, and the heart, at its next systole, throws out not only the normal quantity of blood, but also that which it has received back from the aorta. The arterial system thus receives at each beat the normal quantity of blood *plus* the amount which leaks back into the ventricle after each systole; so that the amount of blood remaining in the aorta and available for passage on to the capillaries is the same as in the normal animal. On this account, after a lesion of the aortic valves has been established, the mean arterial pressure remains the same as before, although the pulse pressure at each heart beat is increased in amplitude. The augmented output by the ventricles naturally involves increased work on the part of their muscular walls, which react to increased work by hypertrophy. The final effect, therefore, is a heart bigger than normal, with hypertrophied and thickened walls, but capable of maintaining an adequate circulation throughout all parts of the body; in other words, complete compensation has taken place.

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INFLUENCE OF TEMPERATURE ON THE HEART. Warming the heart quickens the rhythm, and cold slows it. In the isolated heart beating in normal sequence, the shortening of the contractions of the different cavities with rise of temperature may be ascribed to the quickening of the sinus rhythm. The changes, however, *i.e.* the shortening of the contraction with rise and the lengthening with fall of temperature, are more marked than when the rhythm is changed by direct excitation of the sino-auricular node itself. The increase of rhythm with temperature is almost proportional to the temperature, and over a limited range this proportionality may be linear.* At 40° C. the isolated mammalian heart may beat four times as frequently as 25° C. Warming the heart beyond 44° C. may cause stoppage of the heart or send it into fibrillation.

Similar effects are produced on conduction, both in the heart muscle itself and in the special conducting tissues. Thus, cooling of the heart leads to an increase in the A.V. interval.

The contraction of cardiac muscle is affected in the same way, a rise of temperature lessening the duration both of the contraction and of the refractory period.

As in a skeletal muscle, the slow individual contraction of the heart at low temperatures is more efficient than the quick contraction at high temperatures, *i.e* when the muscle contracts slowly and maintains the intraventricular tension for a long time, less energy is required for the expulsion of a certain volume of blood than at a higher temperature.

INFLUENCE OF THE COMPOSITION OF THE SURROUNDING MEDIUM ON THE HEART MUSCLE. All the cells of the body require a definite osmotic environment. This is equivalent to a 0·7 per cent. sodium chloride solution for the frog's heart, and to a 0·9 per cent. solution for the mammalian heart. As Ringer first showed, the nature of

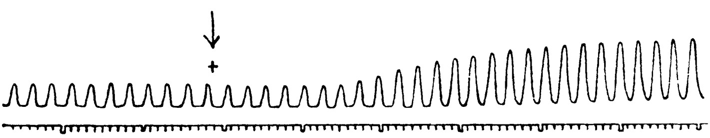

FIG. 366. Tracing of Contractions of a Frog's Heart, showing effect of adding a trace of $CaCl_2$ to the NaCl Solution used previously for Perfusion. (RINGER.)

the salts employed for making up the isotonic solution is all-important to heart muscle.† Thus, a strip of muscle from the apex of the tortoise's ventricle immersed in a 0·7 per cent. solution of sodium chloride, begins to beat rhythmically. The contractions soon reach a maximum and then die away in a condition of relaxation. Sodium chloride therefore acts as a stimulus to contraction, but is unable to maintain the beats for any considerable length of time. On now adding to the solution a trace of calcium chloride the contractions begin again (Fig. 366). The relaxations after each contraction then become more and more incomplete, until finally the heart stops in a tonically contracted condition. If now a trace of potassium

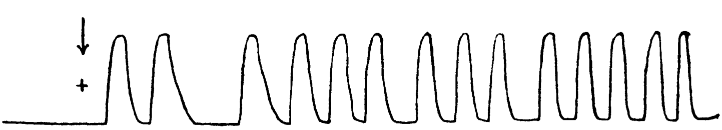

FIG 367. A Frog's Heart, Poisoned by Excess of Calcium Salts, Recovers its Spontaneous Rhythm on adding a trace of KCl to the Perfusion Fluid. (RINGER.)

* KNOWLTON and STARLING. *J. Physiol.*, 1912, **44**, 206.
† RINGER. *J. Physiol.*, 1883, **4**, 29.

chloride be added, the contractions recommence and may last for many hours, although the solution contains nothing which can furnish energy to the contracting muscle (Fig. 367).

The exact significance of the different ions for the functions of cardiac and other forms of muscular tissue must still be regarded as an open question.

Fluids containing the three ions mentioned above in slightly varying proportions, are commonly used to maintain the beat in an excised heart either of a cold- or of a warm-blooded animal (Ringer's fluid, etc., *v.* p. 66). In the case of the latter, it is necessary to keep the fluid warm, and saturated with oxygen. Locke * showed that the addition of glucose to the solutions enables the beats to go on for a longer period of time, and will in fact renew the rhythm of a heart which has ceased beating while being fed with pure saline solution. Addition of sodium lactate is even better.

The influence of the chemical composition of the medium on the contraction of the heart may be investigated in the following ways:

One of the simplest methods of investigating the heart of cold-blooded animals is by perfusing the heart cavities with the fluid under investigation. Two forms of perfusion are made use of. In the method first introduced by Williams a double cannula is tied into the ventricle, the rest of the heart being cut away. The tubes leading to and away from the perfusion cannula are armed with valves so as to allow the fluid to pass only in one direction. The contractions of the ventricle may be recorded either by connecting the outgoing tube with a manometer, or by connecting a piston recorder with the vessel in which the heart is contained, so as to register changes in the volume of the ventricle. A large number of different forms of apparatus has been devised for these purposes.

In another method, the fluid is allowed to flow through the whole heart, passing in by the sinus and out by the aortæ, or, as in Symes' method,† in by a cannula tied in

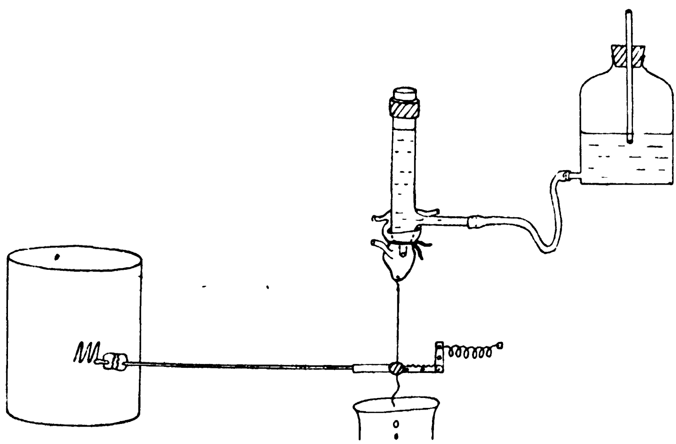

FIG. 368. Symes' Apparatus for Perfusion of Frog's Heart. (HARRIS.)

the auricle, and out by the aortæ (Fig. 368). It is usual to start with Ringer's fluid, and then to omit or increase the constituents one by one, in order to demonstrate their influence.

The heart of warm-blooded animals can also be investigated by a somewhat similar method. It was shown by Porter ‡ that the mammalian heart could be kept alive by perfusing oxygenated blood serum through the coronary vessels, and Locke § found that

* LOCKE. *J. Physiol.*, 1895, **18**, 333.
† SYMES. *J. Physiol.*, 1918, **52**, Proc. xlvi.
‡ PORTER. *Amer. J. Physiol.*, 1898, **1**, 141.
§ LOCKE. *J. Physiol.*, 1904, **31**, Proc. xiii.

the same results could be obtained by using warm oxygenated Ringer's solution of the same tonicity as mammalian blood. A simple apparatus for this purpose is that devised by J. A. Gunn,* and shown in Fig. 369. It consists of an outer jacket, D, through which a constant stream of water at a little above body temperature is passed. The inner tube, F, is made of thin glass, and at its lower end three small indentations, d, d, serve to support a thermometer, T, of such a thickness that only a small space is left between it and the inner tube. Into the lower end of the inner tube the cannula, C, is fixed by a rubber sleeve, while at the upper end a Y-shaped tube delivers, either oxygenated Ringer's solution by B, or any other solution which is to be perfused by A. The proper perfusion pressure is provided by placing the reservoir of Ringer's, or other, solution at the desired height, and it is an advantage to have these reservoirs immersed in a bath kept at body temperature. The heart having been excised and washed well in cold saline, so as to remove as much blood as possible, the cannula is tied into the stump of the aorta, and the perfusion started. The pressure of the fluid closes the aortic valves, so that the solution only passes into the coronary vessels. After passing through the heart, it reaches the right side, and flows away. A bent pin, to which a long thread is tied is hooked into the apex of the heart, and the beats of the heart are recorded by means of a simple lever attached to the thread.

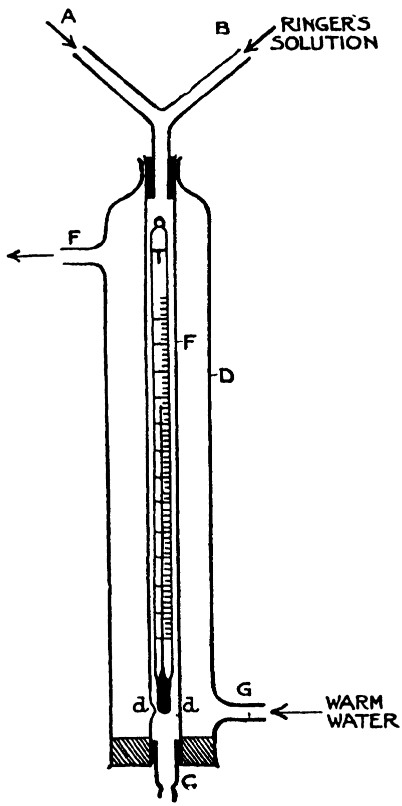

FIG. 369. Gunn's Perfusion Apparatus for the Mammalian Heart.

HYDROGEN ION CONCENTRATION. The reaction of the perfusing fluid has considerable influence on the frog's heart. When weak acids are transfused through this heart, the heart dilates, the beats become smaller, slower, and finally at about pH 2 or 3, disappear.* A similar relaxation may be obtained as the result of the action of carbon dioxide. On perfusing with weak alkalies on the other hand, the relaxation during diastole becomes more and more incomplete, so that the heart is finally arrested at pH 11 or 12 † in a contracted condition. There will thus be some reaction, actually at pH 8·3,‡ which will represent the optimum reaction for the beat of the frog's heart. Mines showed that this optimum reaction is not the same for the different cavities of the heart, nor for the hearts from various animals, but a shifting of the reaction of the perfusing fluid to the acid side always brings about a diminished contraction with dilatation, while the opposite effects are produced by an increase to the alkaline side.

In the mammal under normal conditions, the chief variable factor affecting the reaction of the blood is the tension of carbon dioxide in this fluid; and any change in the carbon dioxide of the blood produces corresponding alterations in the heart's action. The effective factor is probably always the hydrogen ion concentration, so that a deficiency of CO_2 may be partially compensated for by reduction of the $NaHCO_3$, *e.g.* by an increased amount of lactic acid in the blood. The optimum pH is 7·5 to 8·0.

Decreased pH (reduced alkalinity) causes (1) Slowing of the cardiac rhythm from its action on the sino-auricular node. (2) Slower propagation and deficient spread of the excitatory wave through the auricular muscle and the A.V. bundle

* GUNN. *J. Physiol.*, 1913, **46**, 506.
† MINES. *J. Physiol.*, 1913, **46**, 216.
‡ CLARK. *J. Physiol.*, 1913, **47**, 70.

(Drury and Andrus *). (3) Dilatation of the ventricles, which is greater than can be accounted for by the slowing of the heart and the greater filling thereby induced (cp. Fig. 370). (4) Dilatation of coronary vessels.

Yandell Henderson † found that vigorous artificial ventilation of the lungs brought about a condition in which the heart's contraction was very forcible but the heart's cavities almost empty. He ascribed this condition to the hypertonicity of the heart muscle produced by washing the carbon dioxide out of the blood. The condition of low CO_2 content of the blood (and consequent raised pH) is known as '*acapnia*.' Dale and Evans ‡ showed that, in the condition of acapnia there is paralysis of the spinal vasomotor centres and a large fall of arterial pressure. The inflow of blood to the heart is therefore decreased, and the heart, having only a small amount of blood to contract on, looks 'hypertonic,' *i.e.* reduced in size. The conditions may be at once removed by adding 5 per cent. CO_2 to the air used for the artificial ventilation. When the heart is perfused with a saline fluid of very alkaline reaction (pH 8·4) the coronary flow is so

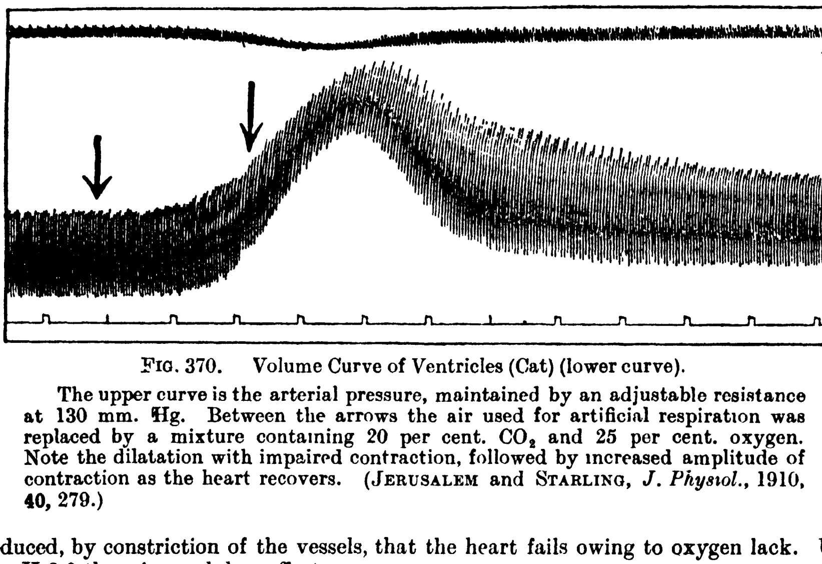

FIG. 370. Volume Curve of Ventricles (Cat) (lower curve).

The upper curve is the arterial pressure, maintained by an adjustable resistance at 130 mm. Hg. Between the arrows the air used for artificial respiration was replaced by a mixture containing 20 per cent. CO_2 and 25 per cent. oxygen. Note the dilatation with impaired contraction, followed by increased amplitude of contraction as the heart recovers. (JERUSALEM and STARLING, *J. Physiol.*, 1910, **40**, 279.)

reduced, by constriction of the vessels, that the heart fails owing to oxygen lack. Up to pH 8·0 there is much less effect.

THE NERVOUS REGULATION OF THE HEART

So far we have considered the action of the heart as a separate organ, but, in order that it may be adapted to the needs of the body as a whole, its automatic mechanism must be subject to the central nervous system, which must be able to affect the heart either by increasing or diminishing its activity. This subjection to the integrative action of the central nervous system is also necessary for the sake of the organ itself; otherwise the uncontrolled response of the heart muscle to changes in its work might result in damage.

The regulation is effected through the intermediation of afferent and efferent nerve fibres connecting the heart with the central nervous system. The afferent supply is the depressor nerve; the efferent, the vagus and sympathetic. They are not essential to life. Thus a dog, in which all the nerves of the heart had been divided, survived the operation for eight months, the pulse rate during the time not having appreciably altered and

* DRURY and ANDRUS. *J. Physiol.*, 1924, **59**, Proc. xli.
† YANDELL HENDERSON. *Amer. J. Physiol.*, 1908–1910 (several papers).
‡ DALE and EVANS. *J. Physiol.*, 1922, **56**, 125.

the animal being in a fair condition of health. But after the operation, he was found less capable of carrying out work, such as running, since the mechanism for adjusting the action of the heart in response to the needs of the muscles had been lost.

THE EFFERENT CARDIAC NERVES

The heart in vertebrates is supplied with efferent nerve fibres from two sources: from the medulla oblongata along the vagus nerve, and from

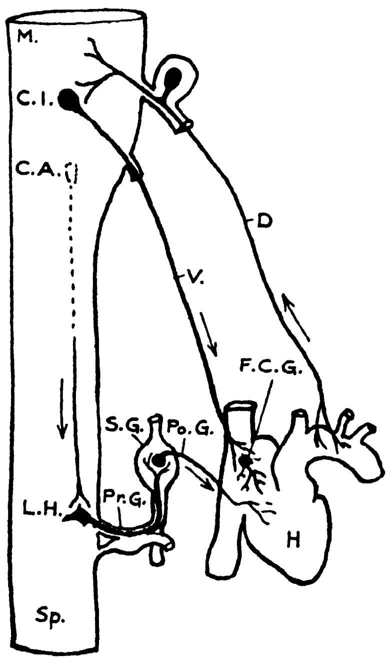

FIG. 371. Diagram of the Innervation of the Heart.

H. Heart.
M. Medulla.
C.I. Cell of cardio-inhibitory centre.
V. Vagus fibre.
F.C.G. Intra-cardiac ganglion cell, where vagus fibre relays.
D. Depressor (aortic) fibre.
Sp. Spinal cord.
L.H. Cell of lateral horn.
Pr.G. Pre-ganglionic sympathetic fibre.
S.G Sympathetic ganglion.
Po.G. Post-ganglionic sympathetic fibre.
C.A. Cell of cardio-accelerator centre.

the upper dorsal region of the spinal cord through the mediation of the sympathetic system (Fig. 371).

THE VAGUS. In the frog, the fibres which leave the vagus to pass to the heart make connection with the cells of Remak's ganglion, and probably with other intrinsic cardiac ganglia, whence non-medullated fibres carry their impulses to the heart muscle. The site of these synapses can be demonstrated by the nicotine method. After painting nicotine on the heart in the region of Remak's ganglion, stimulation of the vagus is without effect on the heart; stimulation of Remak's ganglion or of the fibres leaving it is, however, still effective.

The fibres of the vagus in the mammal arise from the dorsal nucleus of the vagus, and various branches pass to the heart by way of the superficial and deep cardiac plexuses, the anatomical arrangements of which are intricate. In the mammal, the right vagus arborises in the sino-auricular node, and the left nerve in the auriculo-ventricular node.

As in the frog, the vagus fibres relay with ganglion cells at the places named, there being numerous scattered ganglia in both auricles and in the auriculo-ventricular groove, but few, if any, in the ventricles. (Woollard.*) From these nerve cells fibres are distributed to auricles, to the bundle of His and to the base of the ventricles.

THE SYMPATHETIC. The fibres from the sympathetic system take a somewhat different course in the two animals in which the regulation of the heart's activity has been chiefly studied, viz. the frog and the mammal. In the frog (Fig. 372) the sympathetic fibres leave the spinal cord by the anterior root of the third spinal nerve; they then pass through the ramus communicans to the corresponding sympathetic ganglion, whence they run up through the second ganglion and the annulus of Vieussens to the first ganglion; they then pass into the cervical sympathetic strand to the *ganglion trunci vagi*, here they join the vagus and pass down as a vago-sympathetic to the heart.

In most mammals the principal sympathetic fibres leave the spinal cord by the ventral roots of the second and third thoracic nerves, run in white rami communicantes to the stellate ganglion, and thence by the annulus of Vieussens to the inferior cervical ganglion. Cardiac branches convey the sympathetic fibres to the heart, and are given off from the stellate ganglion, the inferior cervical ganglion and the annulus.

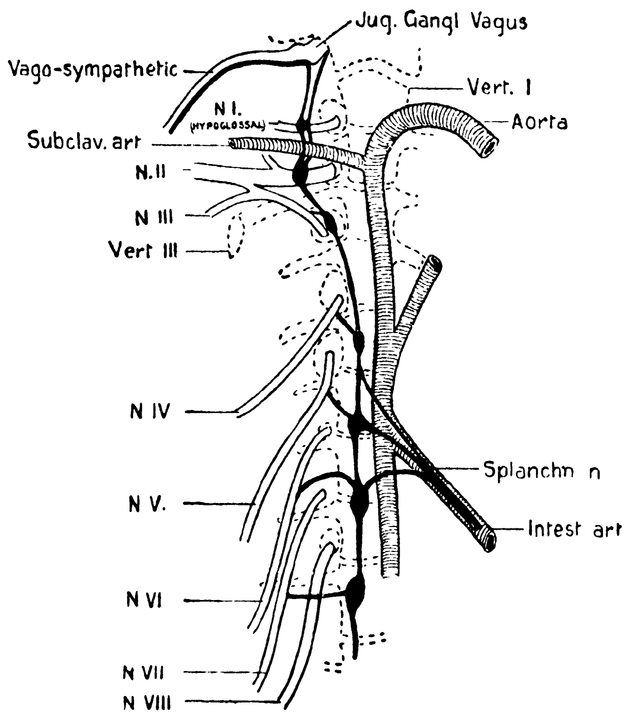

FIG. 372. Sympathetic Chain of Frog (right side) to show Connection with Vagus Nerve.

The sympathetic ganglia with their branches are black. Of the peripheral branches only the splanchnic nerve is represented. (Modified from ECKER.)

These branches run to the superficial and deep cardiac plexuses, in which they cannot be distinguished from the vagus branches.

By the nicotine method it is possible to trace out the cell connections of these fibres. As they leave the cord they are medullated nerve fibres, similar to the other fibres making up the visceral outflow throughout the thoracic region; the white fibres pass along the ramus communicans to the stellate or other sympathetic ganglion, where they end, forming *synapses* with the cells of the ganglion. Here fresh relays of

* WOOLLARD. *J. Anat.*, 1926, 60, 345.

fibres, which are non-medullated, start and carry the impulses to the heart along the various cardiac nerves just mentioned. In the heart these fibres are distributed to the muscle fibres without the intervention of any other ganglion cells.

Although the principal supply of sympathetic fibres to the heart is as stated above, there are subsidiary supplies derived from the white rami of the thoracic spinal nerves down to the 6th or 7th, while the cardiac branches from the sympathetic chain run from the superior, middle and inferior cervical ganglion, as well as from the upper thoracic ganglia, to the heart. This probably applies also to man (Fig. 373), though it is not certain that all these branches are cardio-accelerator in function.

ACTION OF THE VAGUS. The action of the vagus fibres on the heart is almost identical in frog and mammal and is exerted primarily on the auricle. Since the auricle normally drives the ventricle, this also is affected. If in the dog the peripheral end of the cut vagus be stimulated, while the arterial blood pressure is being recorded, diastole is lengthened almost immediately, the pulse is seen to become slower, or, with a stronger stimulus, to cease altogether, and the blood pressure falls towards zero. On discontinuing the stimulus, the heart begins to beat again and the pressure rises after a few beats to normal (Fig. 374).

If the stimulation of the vagus be prolonged, the blood pressure, on discontinuance of the stimulus, may rise above normal owing to the asphyxia of the vaso-motor centres produced by the prolonged cessation of the circulation.

When the arterial system is dilated, so that the mean systemic pressure during cardiac inhibition is low, or when the asphyxial gasps of the animal are prevented by section of the spinal cord, the heart may fail to recover from the inhibition produced by stimulation of the vagus.

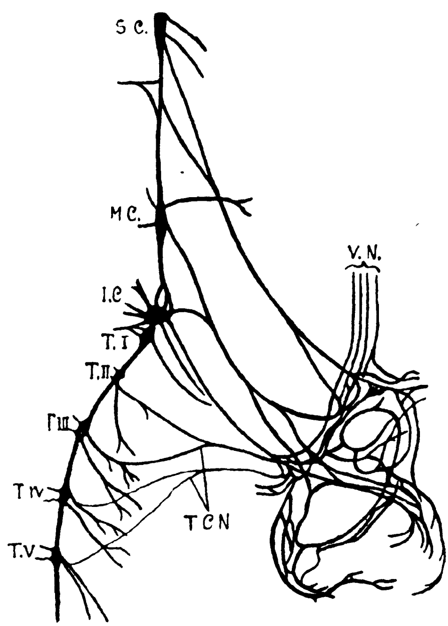

FIG. 373. Diagram of Sympathetic and Vagus Supply to the Cardiac Plexuses (Man). S.C., M.C., I.C., superior, middle and inferior cervical ganglia. T. 1, II, III, IV, V, thoracic sympathetic ganglia. T.C.N., thoracic cardiac nerves. V.N., cardiac branches of vagus. (KUNTZ.)

To study the influence of the vagus on the auricles and ventricles respectively, it is necessary to record separately the contractions of the different chambers of the heart, or the electro-cardiogram may be used as an index to the changes in the heart under the action of nerve stimulation or section. The vagi may affect the heart in one of several ways. Their chief action is on the venous end. Vagus stimulation may cause auricular standstill, slowing, flutter or fibrillation. The right vagus, since it connects with the sino-auricular node, has the greater effect in lowering the rate. If its action is confined to the sino-auricular node, the sole effect of the vagus on the auricles and ventricles will consist in an alteration of rhythm. They may cease to beat altogether, or they may give beats of normal strength but at a slower rhythm than before. Often, indeed, under these conditions the beats of the ventricles may be increased in size, since the strength and

extent of their contractions will be greater with any prolongation of the diastolic period, and consequent increased diastolic filling of the ventricle.

If the vagus (especially the left) acts on the auricles without affecting the sino-auricular node, the rhythm will be unaltered, but the strength and duration of systole, and the refractory period of the auricles, will be diminished. Under such circumstances the rhythm of the ventricles will be unchanged.

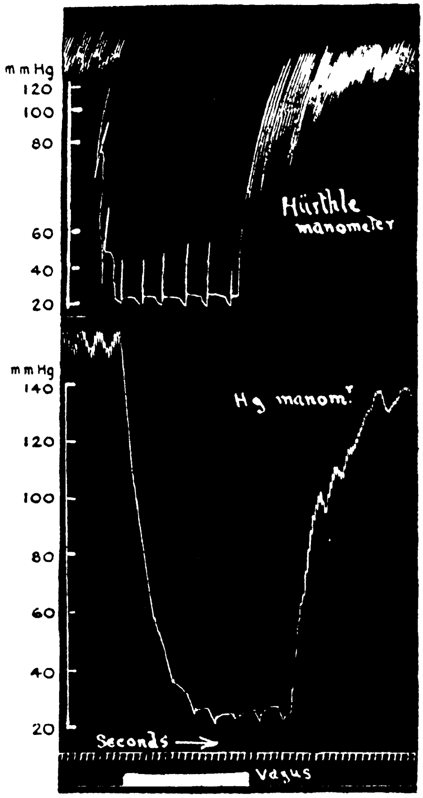

FIG. 374. Blood Pressure Tracing from Carotid of Dog (taken simultaneously with Hurthle's Manometer and Mercury Manometer), showing effect of Excitation of Vagus. (The Mercury Manometer gives very imperfect records of the pulsations during rapid changes of blood pressure.) (C. L. E.)

Generally, strong vagus stimulation stops the action of all parts of the auricles; in such cases the ventricles also cease beating. Very often after a short pause the ventricles commence to beat at a slow ('idioventricular') rhythm. This is called 'vagus escape.' This escape is generally confined to the ventricles, the auricles remaining in a state of diastole. Vagus escape is favoured by distension of the right side of the heart, and is often synchronous with the respiratory efforts which supervene after a certain duration of inhibition, as a result of asphyxia. It appears to be due in part to an accelerating reflex from a distended right auricle,* and from the carotid sinus in which pressure has fallen, and in part to independent contraction of a ventricle temporarily cut off from auricular control. That the ventricle can initiate the escape beat is shown by the fact that occasionally one may observe a contraction of the auricle following, instead of preceding, each ventricular contraction.

Whether the vagus has a direct action on the mammalian ventricle is still doubtful; its effect is, at any rate, very slight as compared with that on the venous end of the heart. The fact that stimulation of the vagus, like division of the bundle of His, usually causes merely temporary cessation of the ventricular beat, would indicate that this nerve has its chief action on the auricles.†

In the frog, it appears that the vagus has a direct inhibitory action on the ventricle, diminishing the strength of its contraction in response to the stimuli transmitted to it from the venous end. This action of the vagus on the ventricle is, however, not found in the tortoise. In both these animals the auricles show the same effects as in the mammal, viz. an influence limited to the rhythm when only the sinus is affected, or a

* McDowall. *J. Physiol.*, 1926, **61**, 131.

† Erlanger and Hirschfelder. *Amer. J. Physiol.*, 1906, **15**, 159; Cullis and Tribe, *J. Physiol.*, 1913, **46**, 141.

diminution of the strength of contraction when the sinus is unaffected and when the chief action of the vagus is on the auricular muscle.

Finally, the vagi may affect the conduction of the excitatory process from auricles to ventricles and to all parts of the ventricles.* Under weak vagus stimulation this is shown by increase in the P-R interval of the electrocardiogram, and in more pronounced effects the ventricles may beat at a slower rhythm than the auricles, a block having been produced in the auriculo-ventricular bundle.

It is evident that these various effects are chiefly due to differences in the place affected. Thus, if the vagus fibres which are distributed to the remains of the sinus are specially active, we shall get alterations of rhythm affecting the whole heart. If those which supply the A.V. bundle are excited, the most pronounced effect will be on the propagation of the excitatory process from auricles to ventricles.

It is remarkable that the excitability of the vagus fibres is dependent on the condition of the cells in the medullary centres. Thus, if the centres are depressed by narcotics, the vagus fibres become more excitable, and similarly if the vagi are sectioned, so that the fibres are cut off from the centres, their excitability steadily increases to a maximum.†

THE TONIC ACTION OF THE VAGUS. If both vagi of a mammal be divided, or if a small dose of atropine be given, the heart as a rule beats more frequently, showing that, under normal circumstances, tonic impulses are constantly descending the vagi and holding the heart's action in check. The extent of the quickening produced by section of the vagi varies in different species and is associated with its powers of carrying out prolonged muscular exertion. Thus, in the dog, hare, or horse the pulse, which is normally slow, may be doubled in frequency by section of the vagi. In the rabbit, which has a frequent pulse, division of both vagi causes very little alteration in the pulse rate. In man, especially in the athletic, vagus tone is high.

This tonic action may be increased by various conditions, *e.g.* by drugs such as morphia. Reflex alteration of the tone may also be induced in various ways to be described later, and is normally the chief cause of alteration in heart rate.

MODE OF ACTION OF VAGUS. Ever since the discovery in 1845 by the brothers E. H. and E. F. Weber ‡ of the action of the vagus on the heart, much work has been expended with a view to determining the intimate nature of the inhibitory process. Atropine is known to paralyse the effect of stimulation of either the vagus trunk or the ganglion of Remak in the frog. It therefore exerts its action at or beyond the post-ganglionic terminations. On the other hand, muscarine or acetyl choline produces the same effect as the stimulation of the inhibitory fibres, and when applied to the isolated auricle or ventricle cause weakening of the beat and finally complete inhibition, this action is also prevented by atropine.

Although various views as to the nature of inhibition have been put forward from time to time, the present tendency is to ascribe both the excitatory and the inhibitory effects of all nervous stimulation to the liberation, at the nerve ending, of small amounts of highly active

* COHN. *J. Exp. Med.*, 1912, **16**, 732; COHN and LEWIS, *J. Exp. Med.*, 1913, **18**, 739.
† GREMELS. *Arch. f. exp. Path.*, 1935, **179**, 360.
‡ E. H. WEBER. *Annali. universali di medicina*, 1845, **116**, 225; E. F. WEBER in Wagner's *Handwörterbuch d. Physiol.*, 1846, **3** (ii), 42.

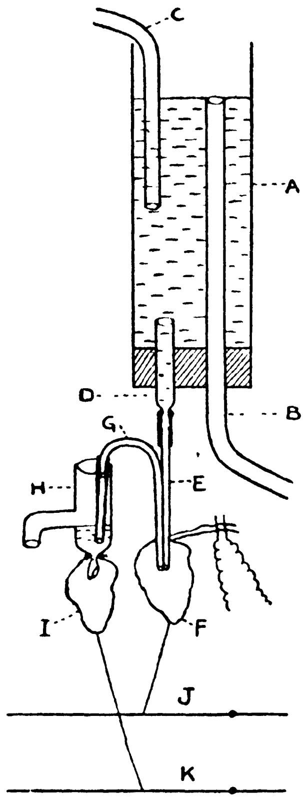

FIG. 375. The fluid from perfusion apparatus A passes to E which supplies the donor heart F. After irrigating the inside of the donor heart, the fluid passes to the glass cannula tube H, to which is attached the recipient heart I. J and K are the levers to which the hearts are attached. (BAIN. *Q. J. Exp. Physiol.*, 1932, **22**, 269.)

substances, which exert their effects on, and are quickly destroyed in, the tissues.

It was shown by Loewi * that the vagus acts by producing a chemical substance which has the power of inhibiting the heart. It is possible to demonstrate, in support of this hypothesis, the 'humoral' transmission of the effect of vagus stimulation. If a frog's heart perfused with saline solution be brought to a standstill by stimulation of the vagus, the fluid if passed on into a fresh heart will produce on the latter the same effect as if the vagus nerve to the second heart were stimulated (Figs. 375 and 376). It is now generally accepted that the mediating substance is acetyl choline, and that the effect of vagus excitation is to produce or liberate this substance in the immediate vicinity of the vagus endings.

The action of acetyl choline is antagonised by atropine, but augmented and prolonged by eserine. The latter effect is due to the fact that eserine paralyses the activity of the enzyme choline esterase, which is prevalent in the blood and tissues, and which normally very rapidly destroys the acetyl choline, so shortening the time over which the effects will last.

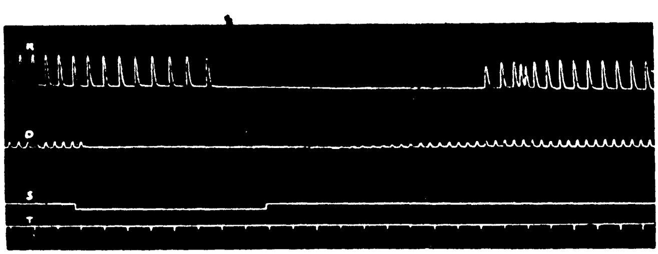

FIG. 376. Tracings from hearts of *Rana esculenta* perfused by method illustrated in Fig. 375. Upper line, recipient heart (R); second line, donor (nerve-stimulated) heart (D); third line, signal; bottom line, time in five-second intervals. Vagus of donor heart stimulated during forty seconds. Arrest of donor heart followed by slowing and then by standstill of recipient heart. (BAIN. *Q. J. Exp. Physiol.*, 1932, **22**, 269.)

* LOEWI. *Pflügers Arch.*, 1921, **189**, 239.

ACTION OF THE SYMPATHETIC CARDIAC NERVES

Stimulation of the sympathetic cardiac nerves at any part of their course has an effect on the heart the reverse of that produced by stimulating the vagi.* In most cases the pulse frequency is increased in consequence of the action of these nerves on the 'pace-maker' of the heart. The frequency attained by maximal stimulation of the accelerator nerves is independent of the previous rate of the heart beat. The increase in rate involves a shortening of the diastolic period. The size of the auricular and ventricular contractions may be increased at the same time as their rate. In fact, like the vagus nerves, the sympathetic fibres of the heart can influence rhythm, strength of contraction, or conduction from auricle to ventricle, according to the part of the heart muscle which is affected.

The augmentor effect on the strength of the ventricular beats is often

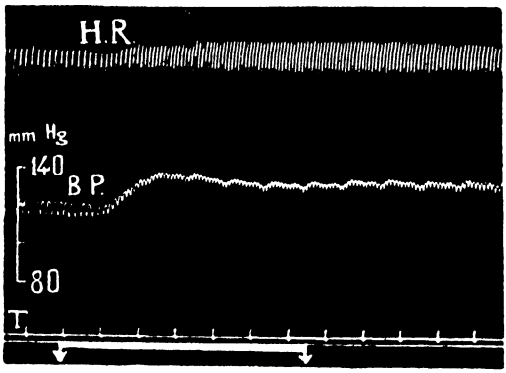

FIG. 377. Heart rate (H.R.) as recorded by Hurthle manometer, and arterial pressure (B.P.) of decerebrate dog, with suprarenals removed and all cardiac nerves cut. Between the arrows stimulation of cardiac nerves from right stellate ganglion causes acceleration of 93 beats per minute and rise of arterial pressure. T = 3-second intervals. Note the latent period of about 6 seconds. (SAMAAN. *J. Physiol.*, 1935, **83**, 332.)

great. The sympathetic fibres are much less easily tired than the vagus fibres, and have a longer latent period. Whereas the latent period of the vagus in the mammal is considerably less than one second, that of the accelerator nerves may amount to ten seconds (Fig. 377). If the vago-sympathetic of the frog be stimulated, the first effect is inhibition due to vagus action. The vagus nerve endings then become fatigued, and the influence of the accelerator fibres makes itself apparent; the heart commences to beat, and the beats become more rapid and forcible than before † (Fig. 378).

Like the vagus, the sympathetic nerve fibres appear to exercise a tonic influence on the heart so that, after extirpation of the stellate ganglion on each side, the pulse may become permanently, though only slightly, slowed.

THE ACTION OF ADRENALINE ON THE HEART. The medulla of the suprarenal glands forms a substance, adrenaline, which has a powerful action both on the heart and blood vessels and plays an important part in the regulation of the circulation. The action of adrenaline on any part of the body is practically identical with that of excitation of the sympathetic nerve supply to the same part. Indeed, as a parallel to the functions of

* CYON and CYON. *Arch. f. Anat. u. Physiol.*, 1867, 389.
† GASKELL. *J. Physiol.*, 1884, **5**, 46; Proc. ix.

acetyl choline in the parasympathetic system, it is probable that the action of the sympathetic nerves is actually due to the liberation of small amounts of

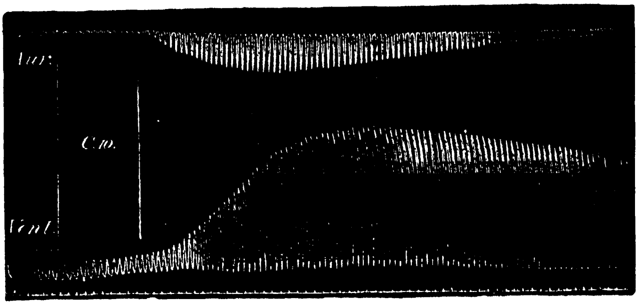

FIG. 378. Tracing to show Effect of Stimulation of the Vago-Sympathetic Nerve on the Frog's Heart. (GASKELL.)

The rhythm is unaltered, but the beats of auricle and ventricle are much decreased in size. On ceasing the stimulation the beats become augmented.

adrenaline at the nerve endings, so that a local effect is produced. Its action on the heart is well shown in the heart-lung preparation. On adding $\frac{1}{10}$ mgm. of this substance to the blood circulating through the preparation, a maximum accelerator and augmentor effect is produced and this lasts for 15 to 20 minutes. Through its influence on the sino-auricular node, the rhythm of the heart is greatly increased, in the dog to about 240 per minute. At the same time the energy of each contraction is augmented.. This is especially shown in a heart which is beginning to fail and is therefore showing

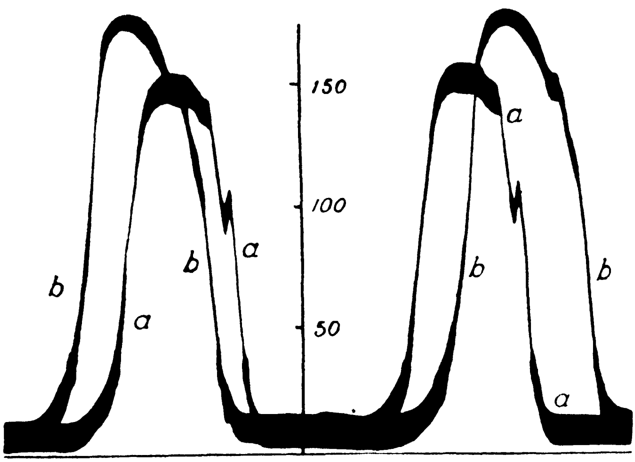

FIG. 379. Intraventricular Pressure Tracings (Left Ventricle) from Dog's Heart (Heart-lung Preparation). (S. W. PATTERSON.)

(To be read from right to left.) The scale shows pressure in mm. Hg.
a. Under influence of adrenaline.
b. Under simultaneous influence of adrenaline and CO_2 (15 per cent.).

dilatation. Directly the adrenaline reaches the heart, the contractions become extremely energetic so that the heart rapidly diminishes in volume, the venous pressure falls, and the blood is thrown out with violence into the aorta. The more powerful beat enables the output of the heart at each

beat to be maintained or even increased, in spite of the shorter duration of the systole. With each beat the maximum pressure in the ventricle therefore rises (*see* Fig. 379). The stimulating effect of adrenaline is shown, moreover, by the fact that the oxygen usage is increased two or three times above that which obtained before the administration of the adrenaline * ; adrenaline exercises at the same time a dilator effect on the coronary vessels, so ensuring an increased supply of blood to the heart muscle. The action of adrenaline, therefore, is to enable the heart to cope with a bigger strain, either in the shape of arterial resistance or increased inflow, than it could otherwise do.

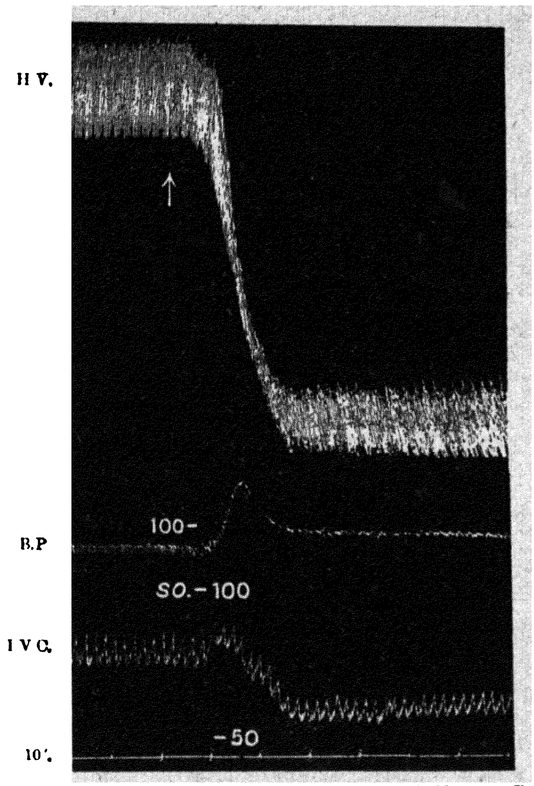

FIG. 380. Effect of Adrenaline on Heart Volume and Venous Pressure. Dog Heart-lung preparation. H.V. = heart volume; B P. = arterial pressure; I.V.C. = venous pressure At arrow adrenaline added to give a concentration of 1 : 3,500,000 in the blood. (PATTERSON, *Proc. R. S.*, 914, **88B**, 3711.)

There is an antagonism in the influence on the heart of the two substances set free during general muscular activity. Carbon dioxide in excess causes dilatation of the heart, diminished activity and slowing of rhythm. Adrenaline causes increased activity, diminution of cardiac volume and increased rhythm (Fig. 380). The action of adrenaline is so pronounced that it is possible to administer 20 or 30 per cent. carbon dioxide to a heart-lung preparation without altering its output, and the beat is maintained and contraction is effective in emptying the heart of its contents if adrenaline be administered at the same time.†

* EVANS and OGAWA. *J. Physiol*, 1914, **47**, 446.
† PATTERSON. *Proc. Roy. Soc.*, 1914, **88B**, 371.

THE HEART REFLEXES

Centres. The part of the central nervous system chiefly concerned in the co-ordination of the various afferent impulses which act on the heart is the medulla oblongata. Here, in the dorsal nucleus of the vagus, we find the *cardio-inhibitory centre, i.e.* nerve cells giving origin to the efferent fibres of the vagus nerves, and also the collection of grey matter in which the afferent fibres of the vagus terminate.* Direct stimulation of the vagus centre may cause slowing or stoppage of the heart. The tonic influence of the vagi is abolished by destruction of this centre. In this region we also find the vasomotor centre, and the two centres are often affected together, though in opposite directions, excitation of the cardio-inhibitory centre for instance being accompanied by a reduced activity of the vasomotor centre. The cardio-inhibitory centre may be played upon by impulses arriving at it through various afferent nerves or from the higher parts of the brain, which give rise to the changes of the pulse rate associated with the emotional conditions, or it may be directly affected by the composition of the blood circulating through its capillaries.

The nerve cells which give off the sympathetic accelerator fibres are situated in the intermedio-lateral tract of the spinal cord, near the roots of origin of these fibres. We might, therefore, speak of a *cardio-accelerator* centre in this region ; but it is most probable that the activity of these cells is subordinated to impulses arriving at them from a common site for control of visceral impulses, viz. the medulla, or even higher, as in the hypothalamic region.

Most sensory nerves of the body when stimulated give either a slowing or a quickening of the heart. Stimulation of the fifth nerve, as in the nasal mucous membrane, sometimes causes reflex inhibition.

In the various reflexes in which these cardiac centres are involved, this happens in a reciprocal manner, *e.g.* increase of action of the accelerator centre being accompanied by a reduced activity of the cardio-inhibitory centre.

The most important afferent nerves which affect the heart reflexly are the cardiac afferents, from the heart itself, and the carotid sinus nerve.

Cardiac Afferents. Among the afferent nerves which can reflexly affect the action of the heart are nerves coming from the heart itself. In the mammalian heart, nerve strands can be seen running over the surface of the ventricle ; these are entirely afferent, stimulation of their peripheral ends causing no effect on the heart beat. Stimulation of their central ends may cause one of five results :

(*a*) Slowing of the heart.
(*b*) Acceleration of the heart.
(*c*) Rise of blood pressure from constriction of the splanchnic area.
(*d*) Fall of blood pressure by dilatation of the arterioles of the body.
(*e*) Reflex movements.

These strands ultimately pass up in the vagus nerves.

The heart does not seem to be provided with nerves of ordinary sensibility. There is no doubt, however, that under abnormal circumstances, impulses arising in the heart can give rise to sensations of pain, which are referred not so much to the heart as to the surface of the body over the left side of the chest and left arm, in the region of the distribution of the cutaneous branches of the second and third dorsal roots. The nervous

* MILLER and BOWMAN. *Amer. J. Physiol.*, 1915, 39, 149.

impulses responsible for these anginal pains have been said to be conveyed by sympathetic afferents.

THE AORTIC NERVE. The chief afferent nerve coming from the heart, or rather mainly from the beginning of the aorta, is the *cardio-aortic*, or *depressor nerve*.* Neither name is quite satisfactory. It is usually bound up with the vagus, but many of its fibres form a separate nerve in the rabbit.

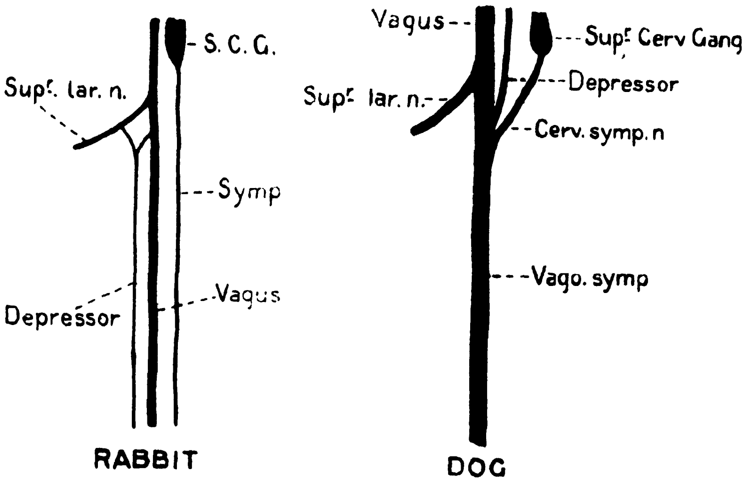

FIG. 381. Diagrams of the Connections of the Depressor Nerve in the Rabbit and Dog, according to Cyon.

This connects with the superior laryngeal branch of the vagus and with the upper vagus trunk, and runs parallel with the vagus to the cardiac plexus

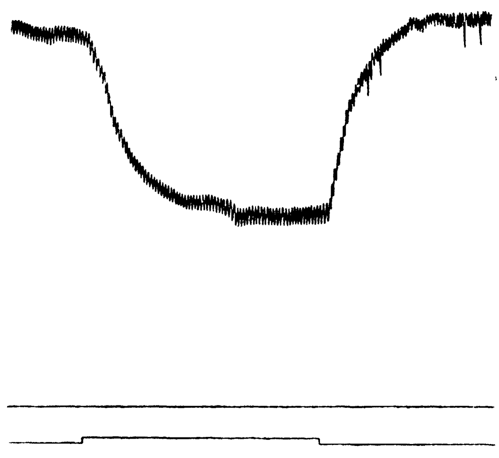

FIG. 382. Blood Pressure Curve from Rabbit, showing effect of Excitation for 17 minutes of Central End of Depressor Nerve (Mercurial Manometer). The middle portion of the tracing, lasting about 12 minutes has been deleted. Time tracing = 12 seconds. (BAYLISS, *J. Physiol.* 1893, 14, 303.)

(Fig. 381). In man it is represented by a branch of the external branch of the superior laryngeal nerve.

* LUDWIG and CYON. *Ber. Sächs. Ges.*, 1866, 307.

The cardio-aortic nerve arises from endings in the aortic arch and in the left ventricle. These endings are sensitive to stretch.

It is purely afferent, stimulation of its peripheral end causing no effect. On stimulating its central end, or the central end of one vagus, preferably the left, a fall of arterial blood pressure (Fig. 382) and reflex slowing of the heart are produced, the latter effect being abolished by section of both vagi. The reflex slowing is due to impulses which ascend by the cardio-aortic fibres of one vagus and are then returned from the vagus centre *viâ* the cardio-inhibitory fibres of the other vagus. The fall of blood pressure still occurs on central vagal excitation, when both vagi are divided. It was shown by Bayliss * that this depressor effect is due to universal dilatation of the blood vessels of the body, chiefly in the splanchnic area.

The aortic nerve is brought into action by stretching or dilating the aorta, as happens when the blood pressure is high. At each systole a rapid shower of nerve impulses can be shown to pass up the afferent fibres of the vagus or depressor; when the aortic pressure is higher these impulses are more numerous. Synchronously with these, a shower of outward impulses passes down the efferent fibres of the vagus. If the depressor nerves be cut when the aortic pressure is high, the blood pressure rises still higher. We have here a means by which the heart can be relieved of a load too great for its powers.

The *aortic body*, a patch of vascular tissue on the aortic lining, and similar in structure and function to the carotid body, also sends fibres to the cardio-aortic nerve. The nerve endings from which these fibres arise are sensitive to chemical changes in the blood, but impulses along them do not affect the heart rate.

OTHER VAGAL AFFERENTS. Some strands of the cardiac plexus cause acceleration of the heart when centrally stimulated. It is probable that these strands originate in the right side of the heart; they reflexly lower vagus tone. Afferent impressions reaching the centres from the lungs *viâ* the vagi also affect the rate of the heart.† Slight inflation of the lungs or weak stimulation of the pulmonary branches of the vagi cause reflex acceleration ‡; great distension, or strong stimulation, causes reflex slowing. (*v.* also p. 628).

THE CAROTID SINUS NERVE. Another important afferent nerve arises in the carotid sinus. This is a specially innervated part of the vessels and tissues in the neighbourhood of the bifurcation of the common carotid into its branches, and includes also the carotid body. In man (see Fig. 383), and many other mammals, the common carotid artery divides into the internal and external carotids, and a bulbous expansion, the carotid sinus or carotid bulb, is seen at the root of the internal branch. A nerve twig runs from the carotid sinus to the glossopharyngeal.

The nerves originating in the carotid sinus are stimulated by stretch, *i.e.* they originate from special stretch receptors in its walls.

The carotid body is richly supplied with sinusoids lined with endothelium and peculiar 'glomus' cells, the latter being abundantly supplied with nerve fibres that join the carotid sinus nerve. The nerve endings in the 'glomus' cells are not sensitive to stretch, but respond to chemical changes in the blood flowing through the sinusoids.

* BAYLISS. *J. Physiol.*, 1893, **14**, 303; 1908, **37**, 264; *Proc. Roy. Soc.*, 1908, **80B**, 339.

† SAALFELD. *Pflüger's Arch.*, 1932, **231**, 33.

‡ ANREP *et al.* *Proc. Roy. Soc.*, 1936, **119B**, 191.

Impulses set up in the carotid sinus and carotid body produce important reflex changes in heart rate, vascular tone and respiration.

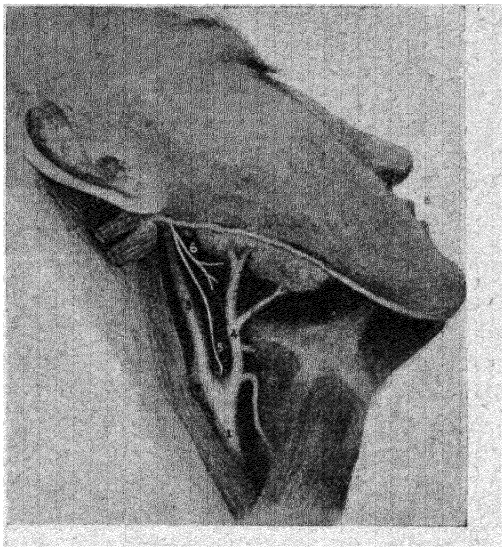

FIG. 383. Showing carotid sinus in man: (1) common carotid; (2) carotid sinus; (3) internal carotid; (4) external carotid; (5) nerve to carotid sinus; (6) glosso-pharyngeal nerve. (C. HEYMANS, "Le sinus carotidien.")

Fig. 384 illustrates diagrammatically the afferent nerve supply responsible for reflex control of the circulation in response to arterial pressure changes,

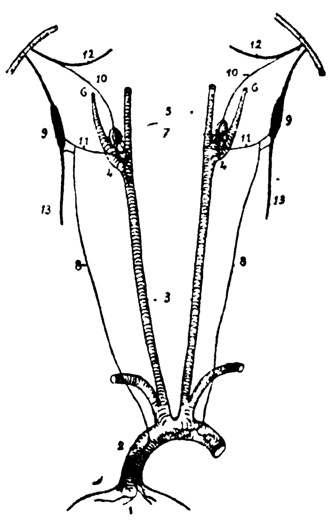

FIG. 384. Scheme of the sensory innervation of the carotid sinus, carotid body and arch of the aorta. (2) Arch of aorta; (3) common carotid; (4) carotid sinus; (5) external carotid; (6) internal carotid; (7) carotid bodies; (8) aortic nerve; (9) ganglion of vagus; (10) branch to carotid sinus joining the glosso-pharyngeal; (11) branch to carotid sinus joining vagus ganglion; (12) glosso-pharyngeal; (13) vagus. (C. HEYMANS.)

i.e. the aortic and carotid sinus innervation. The efferent paths are, of course, the vagus, for cardio-inhibition, and the sympathetic nervous system, connected with the relevant bulbar centres by way of the spinal cord, for cardio-acceleration and, as we shall see later, also for vascular tone.

Stimulation of the carotid sinus electrically, or mechanically, as by stretching its walls by pressure from within in any manner, provokes a combined reflex of cardiac inhibition and fall of blood pressure, just as does stimulation of the central end of the depressor nerve. In fact, we may regard the reflexes from the carotid sinus and those from the aortic nerves as part and parcel of the same physiological arrangement for controlling the blood pressure. It is found that, after section of the vagi, stimulation of the carotid sinus in any way no longer produces the cardiac inhibition (proving that this was reflex).

Chemical Responses. Altered chemical content of the blood in the carotid body can produce alterations in heart rate by exciting the chemo-receptors, *e.g.* increase of CO_2 or deficit of oxygen causes reflex slowing of the heart. The effects are relatively weak, however.

Cardiac Reflexes. There are two very important reflex mechanisms associated with the heart itself. If, other conditions remaining the same, the arterial pressure be raised, the heart is slowed. This phenomenon, sometimes dignified with the name of "Marey's law," * *i.e.* that *the pulse rate varies inversely as the arterial pressure*, is not regularly seen in the intact animal or in man, because other conditions are changed. The other reflex is an acceleration of the heart rate when, other conditions remaining the same, the pressure in the great veins and right auricle is raised, as happens when the venous return to the heart is increased. Thus we have two mechanisms, balanced the one against the other, one causing the heart to slow down if the left ventricle is getting over full, the other causing it to accelerate if the right side distends.

INHIBITORY REFLEXES. The first of these mechanisms in the reflex cardiac inhibition, resulting when afferent fibres of the aortic nerves (chiefly the left) are excited by the stretching of the arch of the aorta and left ventricular wall if the blood pressure rises. As a result of this the cardio-inhibitory centre is excited, with the sequel that efferent cardio-inhibitory impulses *viâ* the vagi bring about a reduction of the pulse rate.

An equally, or even more, important cause of the cardiac inhibition consequent on rise of arterial pressure is a reflex from the carotid sinus.

This is shown by the striking experiments of Heymans, called the "perfusion of the carotid sinus." For this purpose, in a dog (B), the sinus region is isolated from the circulation by ligatures, and left connected with the animal only by its nerves; this isolated segment of carotid artery with its branches can then be perfused with blood from a pump, or from a second dog (A). The blood from the carotid of A enters the cephalic end of the carotid artery of B, and, after passing along the carotid sinus segment, is returned *viâ* the external carotid artery of B to the external jugular vein of A (Fig. 385).

The pressures in the femoral artery of the recipient dog and of the perfusing supply (pump, or artery of donor) are recorded. If now the perfusing pressure is raised in any way, so that pressure is increased in the sinus of dog B, there is an immediate reduction of heart rate and fall of arterial blood pressure in the systemic circulation of the latter. If the blood pressure in the sinus of B is still further increased, there is a still further slowing of the heart and fall of pressure in dog B (Fig. 386). Conversely, by lowering the pressure in the carotid sinus of B, its systemic arterial pressure is raised and there is

* MAREY. *Physiol. médicale de la circulation du sang*, 1863, p. 206 (Paris, Delahaye).

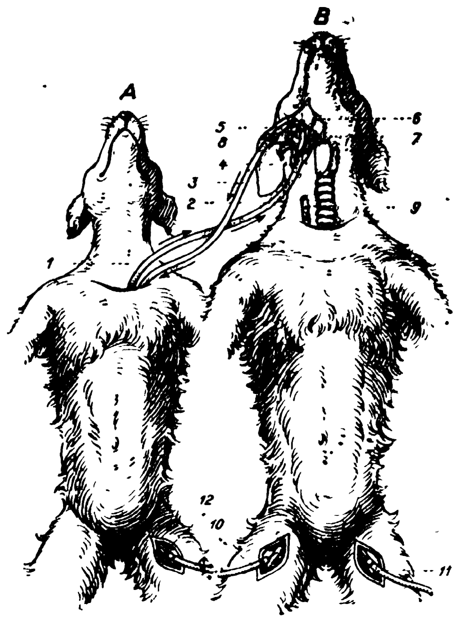

FIG. 385. Perfusion of isolated carotid sinus. A, dog supplying perfusion blood. B, dog of which the sinus is perfused. (1) Carotid artery of dog A ; (3) external jugular of dog A ; (6) nerve supply of carotid sinus of B. All the branches of the carotid sinus of B are tied off, so that the only blood entering it is that from A. (C. HEYMANS.)

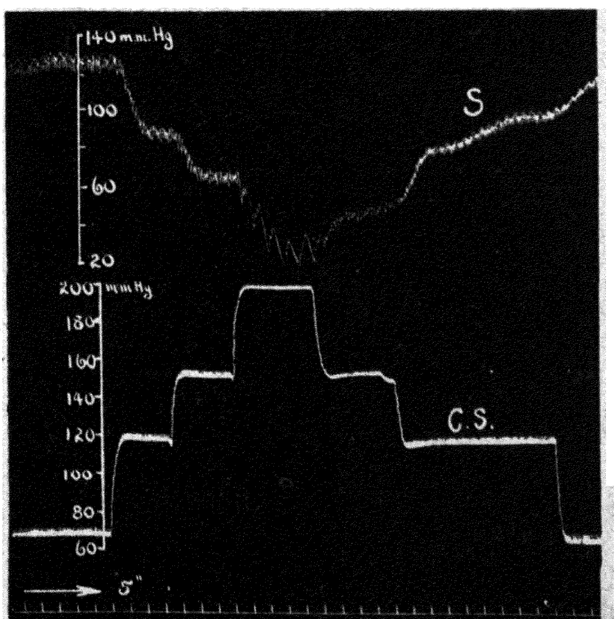

FIG 386. Perfusion of the carotid sinus in the dog. The carotid sinuses are isolated from the systemic circulation and supplied with defibrinated blood by means of a perfusion pump. Upper tracing, S = systemic blood pressure ; lower tracing, C.S. = pressure of blood in carotid sinus. Alteration of pressure in the carotid sinus produces a change in the opposite direction in the systemic blood pressure. (From a tracing by DR. G. STELLA.)

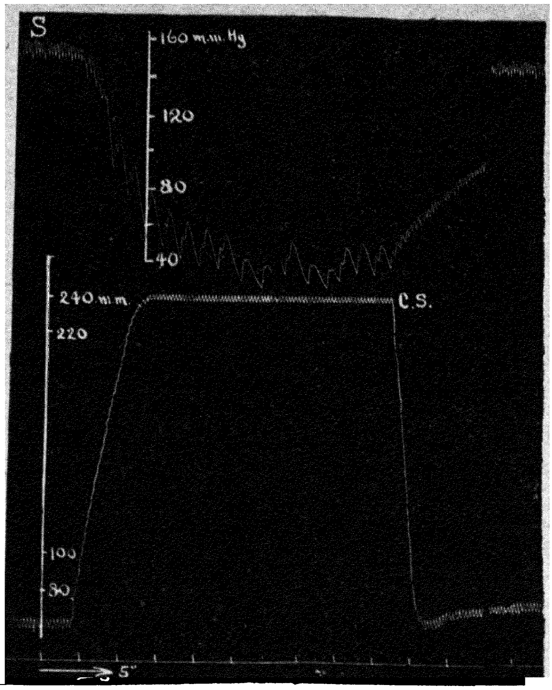

Fig. 387. Showing definite cardiac inhibitions as a result of increasing the pressure in the carotid sinus, which was supplied with blood by a pump, and isolated from the systemic circulation. The break on the right represents an interval of about 30 seconds. (From a tracing by Dr. G. Stella.)

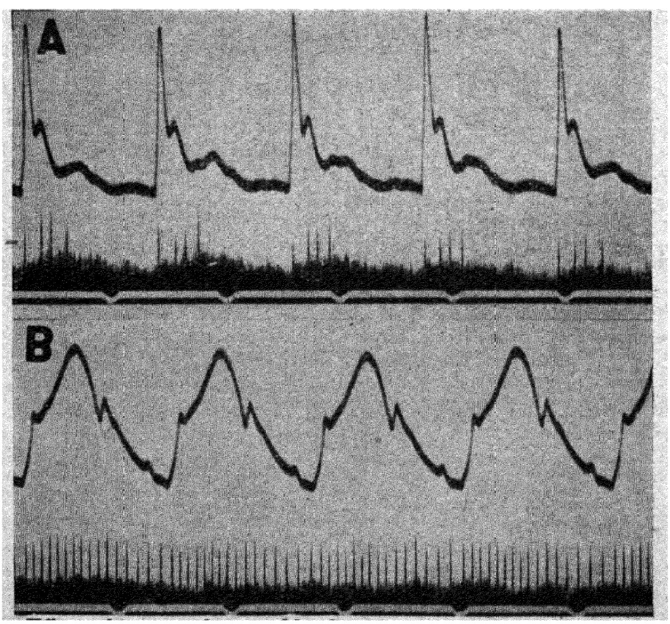

Fig. 388. Effect of increased mean blood pressure on the discharge from a single end-organ of the carotid sinus. In each tracing, the top curve is arterial pressure, middle one is electrical variations in sinus nerve, lowest one = 1/5th second. In A, B.P. = 55 mm. Hg. In B, = 135 mm. Hg. (Bronk and Stella, *Journ. Cell. and Comp. Physiol.*, 1932, I, 113.)

at the same time cardiac acceleration in B (Fig. 387). The alterations of arterial pressure in the dog B, though they accompany the alterations in heart rate, are only in part due to them, since the pressure changes still persist after elimination of the cardiac effects by section of the vagi or administration of atropine (see p. 686).

That nerve impulses along the carotid sinus nerve are set up by pressure changes in the carotid sinus has been beautifully shown by the experiments of Bronk and Stella. They recorded the electrical variations representing the nervous impulses passing along a single nerve fibre from the carotid sinus, as shown in Fig. 388. It will be seen that in tracing A, where the blood pressure was low, a burst of impulses passed along the nerve during the systolic part of each pressure-pulse curve. When the arterial pressure was raised, there was a more rapid stream of impulses, which continued also during the diastolic portion of the curve, though at a slower rate. (B).

ACCELERATOR MECHANISMS. The other mechanism is a reflex, often called the Bainbridge reflex,* which is started from either auricle and from the great veins, when these become distended by rise of venous pressure.† Afferent impulses from the auricles travel up by the vagi (perhaps chiefly by the right one) and bring about reflex cardiac acceleration by (*a*) reducing the tone of the cardio-inhibitory mechanism (the principal factor), and possibly also by (*b*) throwing the cardio-accelerator mechanism into action.

Cardiac acceleration can, of course, also result from reduction of the vagus tone arising from the inhibitory zones, the carotid sinus and aortic arch. This happens when the arterial pressure falls.

EFFECTS OF CHANGES IN CEPHALIC ARTERIAL PRESSURE. An important question which arises out of the discovery of the carotid sinus reflexes is that which concerns the effect on the general circulation of increased arterial pressure in the head. It has been generally agreed that an increase in the intracranial arterial pressure tends to produce, as compensatory phenomena, slowing of the heart and a fall in the somatic blood pressure.

Most investigators believed that the effects were entirely due to the action of increased

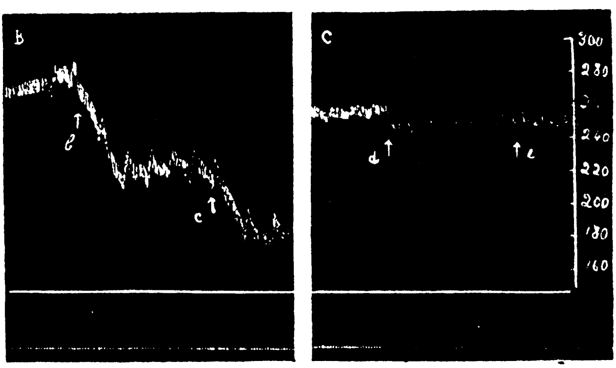

FIG. 389. Showing the effect of carotid sinus reflexes on blood pressure in an intact anæsthetised dog—the record shows femoral blood pressure. B: the carotids are clipped off; at *b*, one clip is removed; at *c*, the second clip is removed. The blood pressure falls from 280–210, and then to 180 mm. mercury and the heart becomes slower. C: after denervation of the carotid regions, removal of the first clip at *d*, and second clip at *e*—no effect. (C. HEYMANS.)

pressure upon the medullary centres, until it was shown by Hering that these effects disappeared when all the nerves of the carotid sinus had been destroyed. This can be illustrated by the simple experiment of lowering the cranial pressure by occluding the carotid arteries (Fig. 389). It will be seen that the effects disappear if the carotid sinus is denervated.

* BAINBRIDGE. *J. Physiol.*, 1915, **50**, 65.

† SASSA and MIYAZAKI. *J. Physiol.*, 1920, **54**, 203.

An application of the technique of the "isolated head," enables this question to be investigated. In this preparation (Fig. 390) the head of dog B is fed exclusively from dog A by anastomoses between both carotids and external jugulars of the two animals—all the tissues of the neck of B, with the excep-

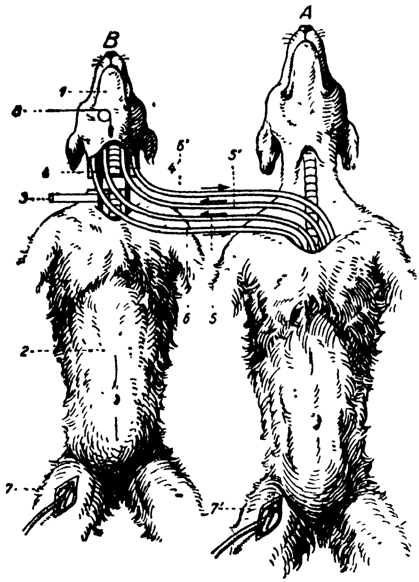

FIG. 390. Schema of the isolated head, according to J F. Heymans. The isolated head of B is supplied with blood from dog A, and is left in connection with its own body only by the two vagus nerves (C HEYMANS)

tion of the two vagi, being divided. The head of B, therefore, is in vascular connection with A, and its only connection with its own body is through the two vagus nerves.

With this preparation it can be shown that a rise of pressure in A leads to a slowing of the heart in B. If the carotid sinuses of B are denervated, alterations of pressure in A no longer produce any effects upon the heart rate of B. We must conclude, therefore, that pressure changes in the head produce their effect upon the heart rate, not through any direct action on the cardio-inhibitory centre, but entirely through reflexes generated from the carotid sinus.

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THE PULSE RATE IN MAN. The normal pulse rate in adult men is about 70 per minute. It is largely influenced by bodily movements and varies considerably with age. The following Table represents the average pulse rate in man at different ages :

Age in years		Pulse rate per minute
0	..	135
5	..	105
10	..	90
15	..	80
20	..	75
25–40	..	70
80	..	75

It must be remembered that marked differences in the pulse rate may be found in different individuals without having any pathological significance. Thus pulse rates of 30 per minute and 120 per minute have been observed in men who were otherwise perfectly healthy. It is slow in athletes. The pulse rate is raised by warmth and diminished by cold applied to the surface of the skin. It is also increased somewhat by the taking of food. The act of swallowing causes a reflex quickening of the rate by inhibition of the tonic vagus action.

The heart-beat is normally not perfectly regular, but shows fluctuations corresponding with the respiratory movements, the rate being accelerated in inspiration and retarded in expiration.

Irregularity of the pulse (or of any other movements) can be conveniently recorded by means of a special instrument devised by Fleisch,* which obviates the labour of measurement of the time intervals on an ordinary tracing. Fig. 391 shows a tracing

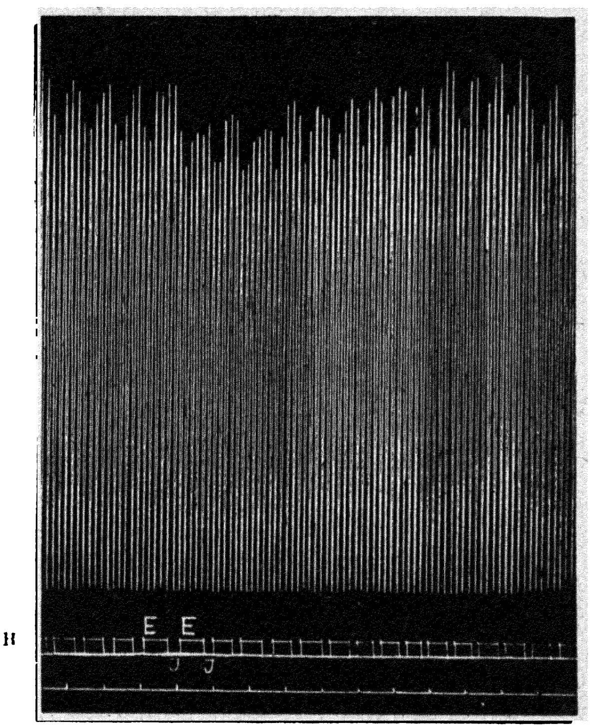

FIG. 391. Normal irregularity of human pulse rate as traced by the "time writer" and "pulse pelotte." The *height* of each vertical stroke gives the time between one pulse and the next. Second line = respiration, E = expiration, I = inspiration. Bottom line = time, six seconds. Variations in rate due to respiration, about 5 pulses per respiratory cycle and also a slower rhythm of change of rate are well shown. (FLEISCH. *Schweiz. med. Wschr.*, 1932, 62, 254.)

made with this apparatus. The height of each stroke on the tracing shows the length of time elapsing between one pulsation and the next, so that a longer stroke means a slower pulse. The tracing shows that at inspiration there is acceleration (shorter stroke), and at expiration retardation (longer stroke); but it also shows, what would not readily be apparent in an ordinary pulse tracing, that there is a slow waxing and waning of the rate, about two or three times a minute. According to Fleisch perfect

* FLEISCH, A. Abderhalden's Hdbch. d. biol. Arbeitsmeth. V. 8, 905: *Zeitschr. f. d. ges. exp. Med.*, 1930, 72, 384; *Zeitschr. f. klin. Med.*, 1932, 119, 618.

regularity of the pulse rate is abnormal. In most normal children, and in certain pathological states, the respiratory fluctuation is so pronounced as to be detectable on mere palpation ; it is called *sinus arrhythmia.* Various explanations have been given for its occurrence, the one recently current being that it is due to a Bainbridge reflex from the right auricle, which is more rapidly filled during inspiration.

The work of Anrep and his pupils,* however, shows that the effect is a complex one, and certainly includes some reflex effects from the lungs and some from the respiratory centre ; these all act upon the vagus centre, increasing or diminishing its tone. When the lungs are inflated, the branches of the pulmonary vagus carry to the vagus centre impulses which reduce its tone ; in deflation these impulses cease. Opposing these reflex effects to some extent are effects of the respiratory centre upon the vagus centre ; thus inflation inhibits the respiratory centre and as a secondary result of this the vagus centre is excited. Hence the effects of the act of respiration, in addition

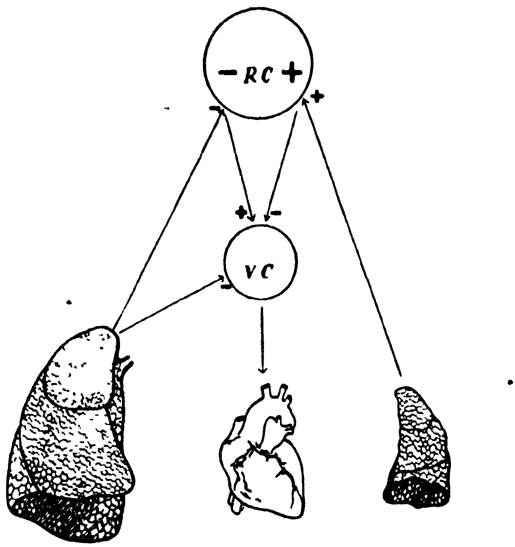

FIG. 392. Diagram showing effects of respiratory act on heart rate. R.C., respiratory centre ; V.C., vagus centre ; on the right, collapse excites R.C , and indirectly inhibits V.C., so tending to produce acceleration. On left, normal inflation does the reverse, but also reflexly inhibits V.C. The effects are a resultant. (Modified from ANREP, PASCUAL and RÖSSLER.)

to that due to the Bainbridge reflex, represent a resultant of excitation and inhibition at each inspiration or expiration. These effects are illustrated in Fig. 392. The influence of respiratory movements is seen in an extreme form in normal persons in Valsalva's experiment. In this, the mouth and nose are closed, and a forced expiration is made. The pulse rate is accelerated, and the arterial pressure raised owing to the great rise in abdominal pressure.†

NUTRITION AND METABOLISM OF THE HEART

THE CORONARY CIRCULATION

In the mammalian heart, the muscle is nourished through the coronary arteries, the venous blood being returned to the right side of the heart by the coronary sinus and other cardiac veins. The branches of the coronary arteries show little anastomosis.‡ Hence, the occlusion of a large branch may lead

* ANREP, PASCUAL and RÖSSLER. *Proc. Roy. Soc.*, 1936, **119B**, 191, 218.
† LEWIS. *J. Physiol.*, 1906, **34**, 391. 1908, **37**, 213.
‡ SCHLESINGER. *Amer. Heart J.*, 1938, **15**, 528.

to serious results. But anastomoses readily develop if one vessel is occluded. Capillaries are very abundant, about 11 m. of capillary per c.mm. of heart muscle.*

The flow of blood through the coronary circulation may be measured by feeding an excised heart, through the coronary arteries, with blood from a pump or heart-lung preparation, and collecting the total outflow from the cut pulmonary artery. Another method is by introducing a cannula through the wall of the right auricle into the coronary sinus and collecting the blood which flows out from the latter. By a comparison of these two methods, it is found, in the dog, that the flow through the coronary sinus usually forms about three-fifths of the total blood passing through the coronary arteries, the remainder passing by Thebesian veins and other lesser channels.† It is therefore possible to measure the flow through the coronary sinus in the heart-lung preparation under varying conditions, and, by multiplying the figures so obtained by $\frac{5}{3}$, to obtain the approximate total flow through the coronary circulation.

Since the efficiency of the heart is ultimately dependent on an adequate supply of oxygen, the importance of the coronary blood flow is evident. About 5 per cent. of the cardiac output is diverted to the coronary circulation. The amount of blood passing through it per gramme of heart per minute may rise to as much as 5 c.c. In man during violent exercise the coronary flow probably amounts to about 1·5 l./min.

Other factors remaining the same, and whether the work of the heart is varied or not, the coronary flow depends primarily on the mean pressure at which the coronary arteries are supplied. The great effect of general arterial pressure on the coronary flow is shown in the following Table :—

Heart weight, 107 grammes. Total Systemic output per minute, 1400 c.c.

Arterial pressure mm.Hg.	Coronary circulation per minute c.c.
60	50
100	90
128	124
166	208
190	500

But this circulation is also adapted to the amount of work that the heart is doing, since the oxygen usage of the heart increases with the amount of work it is given to do. When we investigate the coronary flow in the isolated mammalian heart, as in the heart-lung preparation, we find, accordingly, that it is increased whenever an increase is demanded to supply the necessary oxygen. Hence we find that :—

(*a*) Reduction in the oxygen saturation of the blood passing to the heart soon gives rise to dilatation of the coronary vessels. This dilatation is almost proportional to the degree of anoxæmia, so that, within very considerable limits, it is found that the consumption of oxygen by the heart is independent of the oxygen saturation of the arterial blood. The effect of increasing deoxygenation of the blood on the coronary flow is shown in the accompanying diagram (Fig. 393).

This effect is probably to be explained as due to a direct effect of oxygen lack on the plain muscle of the coronary vessels ; it is seen to occur at once when traces of cyanides are added to the blood, so as to suppress oxygen utilisation (Fig. 393). It has been ascribed to the action of metabolites produced in the heart muscle, and normally eliminated by oxygen.‡

* Wearn. *J. Clin. Invest.*, 1924, **1**, 572.
† Evans and Starling. *J. Physiol.*, 1913, **46**, 413.
‡ Katz and Lindner. *Amer. J. Physiol.*, 1939, **126**, 283.

Carbon dioxide has a dilator influence on the coronary vessels, but this is slight compared with that of anoxæmia, and is probably due to the accompanying slight increase in hydrogen ion concentration.

(*b*) The coronary arteries are richly innervated from both the vagus and the sympathetic, and Anrep and Segall found that the vagal fibres are constrictor to the coronary vessels and normally exhibit a tonic action. Section of the vagi, therefore, not only quickens the heart rate, but also relaxes the coronary vessels and allows an increased flow. Further, when the

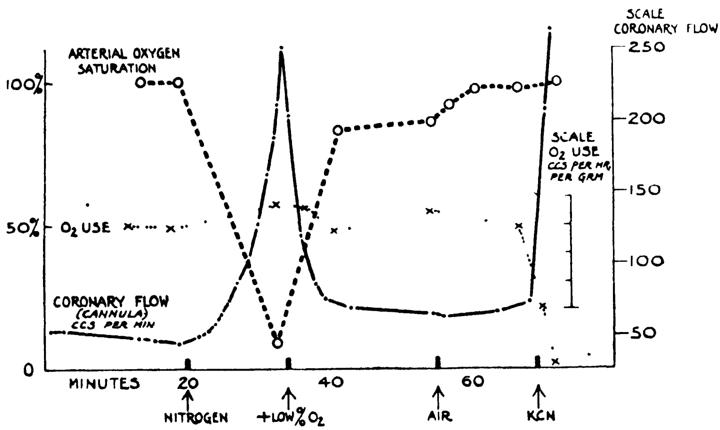

Fig. 393. Curves showing the Relation of the Coronary Blood Flow and of the Oxygen Consumption by the Heart to the Degree of Oxygen Saturation of the Blood passing through the Coronary Vessels. (Hilton and Eichholtz.)

output of the heart in the intact body is increased, there is, in addition to the reflex cardiac accleration, also an independent reflex relaxation of the coronary vessels, whereby the circulation through the heart is increased: this is not exhibited by the denervated heart-lung preparation, in which the effect of alteration of output has but little effect on the coronary flow.

On stimulating the sympathetic nerve supply to the heart, there is an increased frequency and energy of contraction and the same effects are produced by the injection of a small amount of adrenaline into the blood stream. This increased beat is always associated with greatly increased coronary blood flow. It seems, therefore, most likely that the sympathetic nerves to the heart have a direct dilator effect upon the coronary arterioles.* (Contrary to the above views, it is claimed by Katz and Jochim,† from experiments on fibrillating hearts, that the vagus is vasodilator and the sympathetic mainly constrictor, both showing tonic action.) Coronary vaso-constrictor and vaso-dilator reflexes probably enter into many vasomotor reflexes, elicited from almost any part of the body.‡

The above factors may be all involved in the adaptation of the blood flow to the needs of the heart when the activity of this organ is increased, either by increased arterial resistance or by increased output, in both of which the oxygen usage of the heart is increased.

The influence of the various phases of the cardiac cycle upon the coronary flow has been the subject of much debate. Anrep studied the coronary inflow and outflow separately, and found that these do not run parallel.

* Greene. *Amer. J. Physiol.*, 1935, **113**, 361.
† Katz and Jochim. *Amer. J. Physiol.*, 1939, **126**, 395.
‡ Greene. *Amer. J. Physiol.*, 1935, **113**, 399.

Fig. 394 shows the relation of these to other events of the cardiac cycle. The coronary outflow shows three waves, very similar to those seen in the intra-auricular pressure; the most rapid flow is seen during ventricular systole, and is no doubt due to a massaging action of the ventricular contrac-

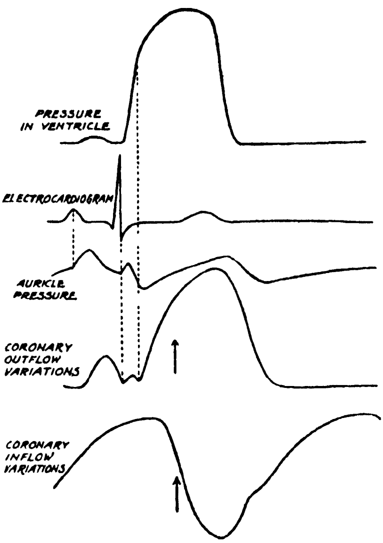

FIG. 394. Relation of Coronary Outflow and Inflow to other events of the Cardiac Cycle. The arrows point in the direction of increase of flow. (ANREP, *et al.*, "Heart." 1927.)

tion expelling blood from the coronary veins. The other waves of the outflow are probably due to the effects of auricular contraction. The variations of coronary inflow in general show a diminution during systole when the hardening ventricular walls press upon the coronary vessels, and an increase in diastole as the external pressure is reduced.

Details are matters of dispute. According to Anrep there is a rapid entry of blood during diastole and early systole, which is suddenly checked as soon as ejection of blood from the left ventricle begins, and reaches its minimum at about the time that the coronary outflow is at its maximum, *i.e.* at the height of the systolic reduction of volume. Wiggers finds that minimal flow is at the end of the ejection phase and maximal at about mid-diastole, with an interpolated rise and fall following the changes in aortic pressure during the ejection phase.*

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WIGGERS (1928). The Pressure Pulses in the Cardiovascular System. (London: Longmans, Green.)

* GREEN, GREGG and WIGGERS. *Amer. J. Physiol.*, 1935. **112**, 627.

METABOLISM OF CARDIAC MUSCLE

The energy for the contraction of cardiac muscle comes ultimately from oxidative processes, and we have seen that the oxygen utilisation of the heart depends on the work to be done, and on its diastolic volume.

When the work of the *isolated* (*i.e.* denervated) heart is increased by raising either the aortic pressure or the output, the oxygen usage rises, and usually the efficiency rises too. The two methods of raising the work in the heart-lung-preparation do not, however, produce the same effects on oxygen usage and hence on efficiency; when work is raised by increase of resistance the oxygen usage shows a greater increase than it does when the work is raised to an equal extent by increase of output. Hence a greater mechanical efficiency results from a given increase of work by increase of output than by increase of resistance. These inferences need to be qualified, however, for the heart *in situ*.

When the work of the heart *in situ* in the normal body is raised, the heart rate is usually altered: *e.g.* when the work is increased by raising the arterial resistance the heart rate is slowed (Marey's reflex), while if it is raised by increase of output the rate is accelerated (Bainbridge reflex). These results involve alterations, in the appropriate direction, of vagus and sympathetic tone. The question as to what happens to oxygen usage and efficiency when the work of the heart *in situ* is raised therefore resolves itself into two subsidiary questions, viz. (1) What is the effect of alteration of rate? (2) What, if any, is the effect of a change of vagus and sympathetic tone apart from that due to change of heart rate?

It is generally admitted that increase of rate leads to an increase, though not a proportionally great one, in metabolism. When the heart is slowed by increase of vagal tone there is a greater reduction in the oxygen usage than could be accounted for by the reduction in number of beats, and conversely increase of the sympathetic tone (or the action of adrenaline) causes a disproportionately great increase in metabolism. Hence it was found that, owing to the increased vagal tone, increase of work due to rise of arterial pressure in the innervated heart was often effected without any increase of oxygen usage at all, and so the heart worked *much more efficiently at the higher load than at the lower.* This provided a marked contrast to the denervated heart, which was very well seen in some cases where for some reason slowing did not occur. The adjustment is called the "unloading reflex."

A further contrast is seen when comparison is made between increase of work by increased resistance and increased output respectively, *e.g.* in the following example the work was doubled:—

Method of Increasing Work	Ao Pressure mm Hg	Output c c /min	Rate per min	Work kg -m /min	O_2 used c c /min	Efficiency Per cent.
Denervated Heart (Evans and Matsuoka, 1914)						
Pressure raised .	40	282	130	0·18	1 63	5 5
	80	280	130	0 34	1 98	8·4
	160	280	130	0 68	4·16	7 9
Output raised .	80	282	130	0 35	2·33	7·3
	80	562	130	0·78	2 64	14·2
	80	1,350	130	2·91	4 96	28·2
Innervated Heart (Gollwitzer-Meier *et al*, 1938)						
Pressure raised .	71	550	125	0·62	3·5	8·8
	136	576	97	1·25	3·2	19·5
Output raised .	60	630	154	0·60	3·8	7·9
	63	1,128	156	1·28	5·4	11·8

These results teach us two things. First, that it is a property of cardiac muscle *per se* to respond by a greater increase of metabolism to rise of arterial pressure than to increase of output and to reach the limits of its performance earlier in the former case than in the latter. Secondly, that when provided with its normal innervation, so that cardiac reflexes are operative, this property is so modified that rises of arterial pressure can be dealt with at least as economically as increases of output.

The cardiac metabolism also depends on the temperature, and on the influence of certain chemical agencies. Thus, if adrenaline is present there is a large increase in the oxygen usage of the heart, which is made possible by the accompanying augmentation in the coronary blood flow. The following experiment illustrates this effect :—

EFFECT OF ADRENALINE 0·2 MG. ON HEART-LUNG PREPARATION OF DOG. (EVANS and OGAWA. *J. Physiol.*, 1914, **47**, 446.)

Heart 58·5 g ; art. press. = 100 mm. Hg ; output c̄. 10·5 l./hr.

	Period of Observation mins	Average Heart Rate p m	C c p hour by H -L P.		R Q.
			O_2.	CO_2.	
Before adrenaline .	80	145	202	164	0·81
After ,, .	30	218	597	463	0·78

A small dose of adrenaline may enable either a normal or a hypodynamic heart to perform tasks previously too great for it. The heart volume is reduced and much greater pressures and volumes of blood can be dealt with. But as the oxygen usage shows, the effect, at least on the *denervated heart*, is not economical but profligate. The heart has been flogged to inordinate activity, and although the work has been done the cost has been high and the efficiency correspondingly low. In fact, by prolonging the administration, the heart works apparently well for a time, and then, with scarcely any warning, passes into acute failure.

When adrenaline is given to an *innervated heart* connected by its nerves to a separately perfused head, the " unloading reflex " already mentioned is brought into play, the increased vagus tone opposing the stimulating effect of adrenaline on the cardiac metabolism, which shows a sharp peak followed by a fall ; the coronary flow is increased greatly so that the venous blood is never very reduced.

Adrenaline also exerts an effect owing to its action on centres in the brain. To study the central effect of adrenaline, the drug is given to the perfused head of an innervated heart-lung preparation, but not to the heart itself. The result is great reduction of metabolism, slowing, and transitory coronary constriction.

Finally, when adrenaline is given to the whole animal we have both types of action— the unloading reflex from the heart itself and the central unloading reflex, both of which antagonise the direct effect of adrenaline on the heart. As the outcome of the competition between them there usually results an actual *diminution* of oxygen usage and of rate, while the work done may even increase. Coronary flow is greatly augmented, and the coefficient of oxygen utilisation is much reduced, because the direct effect of adrenaline on the coronary vessels is greater than the reflex constrictor effect. The efficiency is greatly increased, as contrasted with the lowered efficiency in the isolated heart.

In chemical composition the heart muscle somewhat resembles striated muscle ; it contains glycogen, adenyl pyrophosphate, and creatine phosphate, though the latter is in smaller amount than in striated muscle. It would be expected, therefore, that it might resemble striated muscle in the nature of the chemical reactions associated with contraction, but, in fact, there are certain clear differences.

The metabolic changes in the heart can conveniently be studied by

analysis at intervals of the blood in the heart-lung preparation, and this has often been done. But it has the drawback that the metabolism of the lungs cause changes in the blood which are by no means negligible; the most conspicuous change introduced by the lungs is a great acceleration of glycolysis, by which the blood glucose is converted into lactic acid in passing through the lungs.*

In order to eliminate the lungs, and so obtain a clearer picture of the sugar usage and metabolism of the blood-fed heart doing work, the lungs can be replaced by an artificial oxygenator. Such a heart-oxygenator preparation,† which has many other applications also, is shown in Fig. 395.

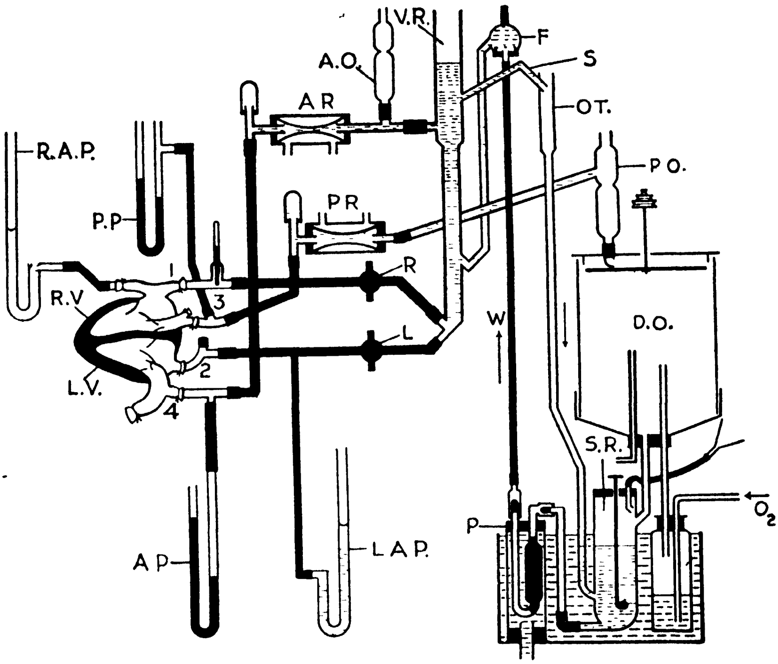

FIG. 395. Heart-oxygenator preparation circuit of EVANS, GRANDE and HSU. (Drawing by courtesy by Dr YULE BOGUE)

The two auricles are supplied with blood through the adjustable taps R and L, and cannulæ 1 and 2, from a narrow common reservoir V.R. This reservoir is kept full to overflowing with blood from a stock reservoir, S.R, by the pump P, the blood passing on its way through a warming tube W and a filter F. The unwanted overflow from V.R. passes by the siphon S along the overflow tube O.T., back again to the stock reservoir. Blood expelled from the aorta passes by cannula 4 through an adjustable pneumatic resistance A.R. (as used for a heart-lung) back into V.R.; the rate of output can be measured when required in the graduated vessel A.O. (Not all the blood expelled by the left ventricle passes out by cannula 4, because the coronary supply has been deducted from it). Blood from the right ventricle (which consists of that entering from cannula 1 plus that returning from cardiac veins) passes through the variable resistance P.R. (which can be omitted), through the graduated vessel P.O, and on to the spinning plate of a Drinker oxygenator, D.O. This is filled with suitable gas, generally oxygen containing 5 per cent. CO_2, and the blood flowing down its sides becomes arterialised and enters the stock reservoir S.R. again. Auricular and arterial pressures are given by the four manometers shown in the figure.

With this preparation the isolated heart can perform work at reasonable rates for

* EVANS, HSU and KOSAKA. *J. Physiol.*, 1934, **82**, 41.
† EVANS, GRANDE and HSU. *Q. J. Exp. Physiol.*, 1934, **24**, 283.

several hours, the conditions of pressure and output on the two sides being independently adjustable. It will also be evident that by closing tap R, the only blood entering and leaving the right side will be the coronary venous blood, the rate of flow or chemical composition of which can thus easily be determined.

It can be calculated that if the heart were only oxidising its own glycogen as a source of energy and not replacing it, a dog's heart containing about 0·7 per cent. glycogen should only be able to work for at most two hours, before exhausting all its glycogen. Since the isolated heart easily works for four or five hours, at the end of which time it often contains an amount of glycogen normal for a fresh heart, it is evident that it either does not normally use glycogen, or else that the glycogen is replaced as used. Now it has long been known that the heart can remove sugar from the blood, but it has recently been shown that the amount used under ordinary conditions is too small to supply the energy requirements.* It does, however, quite unlike skeletal muscle, readily use lactic acid from the blood.*† It also easily consumes pyruvic acid.‡ The amounts of glucose and lactate used vary reciprocally, so that if lactate is absent, glucose is used in greater amount, and *vice versâ*. The ordinary dog heart, in a heart-oxygenator circuit uses about 70 mg. glucose and 200 mg. lactic acid per 100 g./hour,† but if the work is increased the amounts of both are greater.§

The lactic acid used by the heart is normally formed from blood glucose, by glycolysis in the blood stream, this process being accelerated in passage of the blood through the lungs. If, in a heart-oxygenator circuit, the heart is allowed to use up all the glucose and lactate, or if it is subjected to intense work, it then draws upon its own glycogen. It should be noted that in the absence of lactate from the blood, the heart becomes weak, but is restored to a better condition by the addition of lactate, which is more effective for the purpose than is glucose. The glycogen of heart muscle is formed from blood glucose, but not from lactate.‖

In addition to carbohydrate and lactic acid, the heart muscle probably utilises fat; its respiratory quotient is about 0·8 to 0·85. It is uncertain at present whether the heart draws on its glycogen normally, and continually replaces it from lactate and glucose, or whether it normally burns these two substances direct, keeping the glycogen in reserve for emergencies. In oxygen lack, lactic acid is formed by the heart, and glycogen is lost.

Further light is thrown on the chemistry of cardiac muscle by the study of the effect of oxygen lack and of iodoacetic acid. It will be recalled that when a skeletal muscle is poisoned with iodoacetic acid, no lactic acid is formed on contraction, but that contractions can be repeated in presence or absence of oxygen until all the phosphagen has been broken down.

The heart, poisoned with iodoacetic acid can contract for an indefinitely long time if oxygen is available ¶; its usage of lactate is unaltered and its phosphagen content not reduced. When oxygen is withdrawn from it, however, it fails after a very few beats ** (Fig. 396). We may provisionally interpret these results as follows: The energy for contraction is given immediately or mediately, by the breakdown of phosphagen; the energy for resynthesising the phosphagen is provided normally by the *oxidation* of lactic acid. If oxygen is lacking, however, glycogen is broken down instead

* McGinty and Miller. *Amer. J. Physiol.*, 1933, **103**, 712.
† Evans, Grande and Hsu. *Q. J. Exp. Physiol.*, 1935, **24**, 348.
‡ Braun-Menendez, Chute and Gregory. *Quart. J. Exp. Physiol.*, 1939, **29**, 91.
§ Bogue, Evans, Grande and Hsu. *Q. J. Exp. Physiol.*, 1935, **25**, 213.
‖ Bogue, Evans and Gregory. *Quart. J. Exp. Physiol.*, 1937, **27**, 27.
¶ Clark, Eggleton & Eggleton. *J. Physiol.*, 1931, **72**, 25.P.
** Clark. *Q. J. Exp. Physiol.*, 1935, **25**, 213.

(as is normal in all cases for skeletal muscle), with formation of lactic acid, and this change now provides the necessary energy. When poisoned with iodoacetic acid, formation of lactic acid from glycogen is no longer possible; hence, withdrawal of oxygen leads to rapid failure of the heart.

It seems that this oxidation of lactic acid represents an essential aspect of the metabolism of the heart. If the lactate is present in abundance in the blood it is that which is burned. When adrenaline is given the lactate demand of the heart is greatly increased. If lactate is lacking from the blood, then it must be obtained by breakdown of the heart's own glycogen. This,

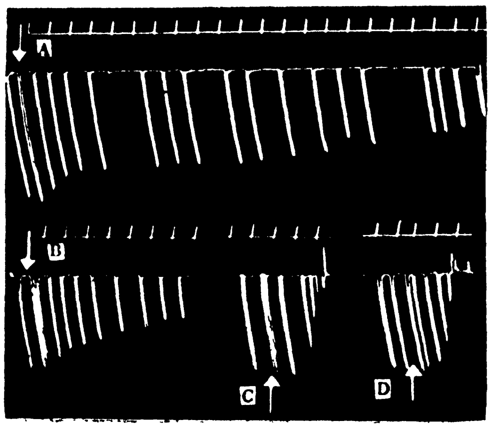

FIG 396. Illustrating the effect of asphyxia on the frog's heart. Isometric contractions were taken for short periods at irregular intervals—downstroke = systole, time = minutes. The arrow shows commencement of full anaerobiosis.
A. Normal ventricle filled 0·3 c.c. of equal parts plasma and Ringer's fluid.
B. Normal ventricle filled 0·3 c.c. unbuffered Ringer's fluid.
C. Ventricle after poisoning with iodoacetic acid, 1 part in 20,000. Filling as in B.
D. Ventricle poisoned iodoacetic acid filled with 2 c.c. equal parts plasma and Ringer's fluid. (CLARK, *Quart. J. Exp, Physiol.*, 1935, **25**, 167.)

however, is either at once, or later on, replaced from blood sugar if any is available; but if not, the glycogen will continue to fall until, when it is all, or nearly all, exhausted the heart fails.

This theory, that oxidation of lactic acid is the central point of cardiac metabolism, explains why lactate cannot form glycogen, but why when abundant in the blood it can prevent glycogen loss.

It may well be that the fundamental difference between cardiac and skeletal muscle is to be sought in two structural differences. First the blood supply of cardiac muscle is relatively greater, and secondly the cardiac muscle cells have no sarcolemma. The first provides for a much more abundant oxygen supply, thereby facilitating all oxidative arrangements; the second enables lactate to pass readily into or out of the muscle cell and so, even if present in the blood, to be readily available for the latter.

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CHAPTER XXXIII

DYNAMICS OF THE CIRCULATION

BLOOD PRESSURE

SINCE the blood travels from the arteries to the veins, it is evident that the pressure which drives it onwards must get less and less as it passes along.

On cutting through an artery, blood escapes from the central end, *i.e.* that nearest the heart, with great force and in a series of spurts, each of which corresponds to a contraction of the ventricles. This shows that in the arteries the blood is at a high pressure, and that the flow from the heart is a pulsatory one.

The same lesson was learnt by an experiment, which was first performed in 1732 by the Rev. Stephen Hales,* and may be described in his own words :

"In December I caused a mare to be tied down alive on her back. Having laid open the left crural Artery about three inches from her belly, I inserted into it a brass Pipe, whose bore was one sixth of an inch in diameter; and to that, by means of another brass Pipe which was fitly adapted to it, I fixed a glass Tube, of nearly the same diameter, which was nine feet in length: Then untying the Ligature on the Artery, the blood rose in the Tube eight feet three inches perpendicular above the level of the left Ventricle of the heart: But it did not attain to its full height at once; it rushed up about half way in an instant, and afterwards gradually at each Pulse twelve, eight, six, four, two, and sometimes one inch: When it was at its full height, it would rise and fall at and after each Pulse two, three, or four inches

Arterial and venous blood pressures are now measured by special manometers, arterial pressures being recorded in terms of the pressure of a column of mercury.

If a vein be ligatured, it swells up on the distal side of the ligature. If the vein be cut across, blood escapes chiefly from the peripheral end, and instead of spurting out with each heart beat, it flows steadily and with very little force, so that light pressure by a bandage is sufficient to restrain the hæmorrhage. If a mercurial manometer be connected with the peripheral end of a vein, the pressure in its interior is found to amount to only a few millimetres Hg. It is therefore customary to use manometers filled with an aqueous solution, *e.g.* sodium citrate, for venous pressure experiments.

By taking the pressure at different parts of the circulation, we obtain a distribution which is represented roughly in Fig. 324, p. 556. The blood pressure, which is about 100 to 120 mm. Hg. in the large arteries near the heart, falls only slowly in these arteries, so that in the radial artery it is not very much below that in the aorta. Between the medium-sized arteries and capillaries there is a very extensive fall of pressure as the blood passes through the arterioles, so that in the capillaries the pressure on an average may be taken as 10 to 20 mm. Hg. ; from the capillaries to the veins the blood pressure falls steadily until in the big veins near the heart it may be "negative," i.e., below atmospheric pressure.

The following Table may serve to give an idea of the average height

* HALES. "Statical Essays ; Hæmastatics, etc.," 1769, (London), 2, p. 1.

of the *mean* (not systolic) blood pressure at different parts of the vascular system in a young adult man, in the horizontal position. The pressures are all subject to considerable variations according to the activity of the individual and of the various parts and organs of the body:

Large arteries (*e.g.* carotid)	. . . . .	90 mm. mercury (65–110).
Medium arteries (*e.g.* radial)	. . . . .	85 mm. „
Capillaries	. . . . . . about	10 to 30 mm. mercury.
Small veins of arm	. . . . . .	9 mm. mercury.
Portal vein (estimated)	. . . . . .	10 mm. „
Inferior vena cava (estimated)	. . . . .	3 mm. „
Large veins of neck	. . . from	0 to –8 mm. mercury.

The Arterial Pressure. The *mercurial manometer*, used for measuring and recording arterial pressure, consists of a U-tube with two vertical limbs about a foot in height, which is half-filled with mercury (Fig. 397). On the surface of the mercury of one limb is a float of vulcanite from which a stiff fine rod rises, bearing on its upper end the writing-point which writes on the travelling paper surface. Since the mercury is displaced equally, up in one limb and down in the other, it is obvious that any displacement as recorded must be multiplied by two to obtain the actual pressure in terms of a mercury column. The other limb of the manometer is connected by a side-branch and a flexible inextensible tube with a cannula, which is tied into the central end of an artery, a clip being previously placed on the artery so as to prevent the escape of blood. This limb is also connected, through a tap, with a reservoir bottle placed several feet above it, and filled with sodium citrate (1 per cent.) or with a half-saturated solution of sodium sulphate. First, the tap is opened and the manometer, connecting-tube and cannula filled with this solution. The tap is then closed so that the manometer remains in connection only with the cannula, the mercury in that limb being now, say, about 150 mm. above that in the other. The clip is then taken off the artery. The pressure in the cannula being greater than that in the artery, a small amount of the fluid used to fill the tubes runs into the circulation. The mercury in the manometer drops to a height, usually of 100 to 120 mm. Hg., and stays about that level, rising and falling slightly with each heart beat (Fig. 398). The blood which enters the cannula at each heart beat does not clot for a considerable time, owing to its admixture with the saline fluid used for filling the cannula and connecting tubes.

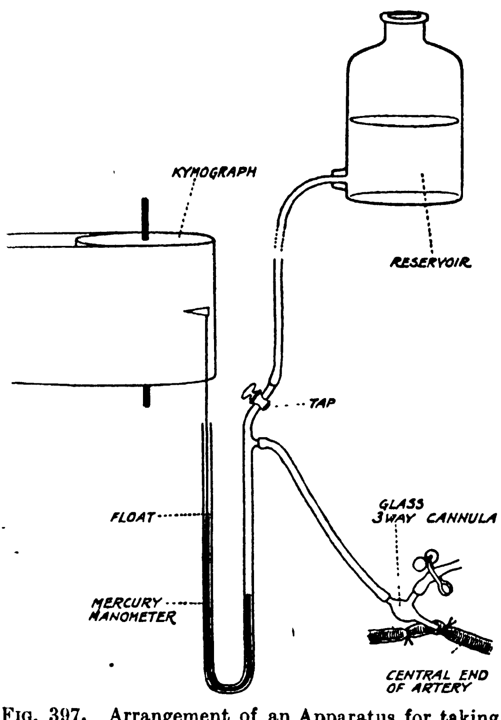

FIG. 397. Arrangement of an Apparatus for taking Blood Pressure Tracing.

The arterial blood pressure as recorded by a mercurial manometer exhibits a series of pulsations, each corresponding to a heart beat (Fig. 398). These pulsations are due to the fact that at each systole in man at least 60 c.c. of blood is pumped out through the aortic valve, in about one-fifth

second, so that the artery becomes distended. During diastole, the aortic valves are closed, and blood passes on from the distended arteries into the capillaries and veins, so that the blood pressure falls. The mercurial manometer, however, owing to the inertia of the column of mercury, does not accurately register these rapid changes of pressure in the artery. With a very wide-bore manometer, in fact, the oscillations may be almost

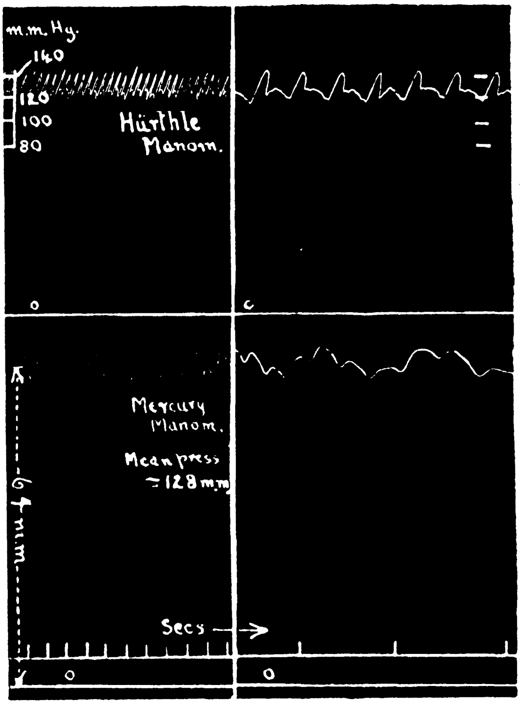

FIG. 398. Arterial Blood Pressure from carotids of Dog, recorded simultaneously with Hürthle Manometer (upper tracing) and Mercury Manometer (lower tracing), on slowly and rapidly moving surfaces. The form and extent of each pulse is much more correctly shown by the Hurthle Manometer, but the slower changes due to respiration are more evident with the Mercury Manometer. 0 = line of zero pressure. The left-hand half is on a slowly moving, and right hand on rapidly moving surface. (C. L. E.)

imperceptible. But it gives a true and valuable record of what is known as the *mean arterial pressure.*

In order to determine the true course of the pressure changes in the arteries, it is necessary to diminish to the utmost extent the inertia of the moving parts of the recording instrument, as in Hürthle's or Frank's membrane manometer, or, better still, one of the forms of manometer in which the pressure changes are registered photographically. Frank's membrane manometer (Fig. 399) is more accurate than a mercury manometer because its period of free vibration is short, so that it readily follows rapid changes of pressure.* It consists of a small tambour, covered with a thick rubber membrane and filled with fluid. It is connected to the artery by an inextensible tube also completely full of fluid (sodium sulphate or citrate solution), and is calibrated by comparison with a mercury manometer. The movements of the rubber membrane are communicated to a light lever, the free period of which is reduced by a spring. The photographically

* FRANK. *Z. f. Biol.*, 1908, 50, 309; 1925, 82, 49.

recording modifications of this instrument have been described in connection with the heart. A further improvement is a manometer with a membrane of thin silver sheet.*

Examination of records obtained by these superior methods (Fig. 398) shows that the variation in the arterial pressure at each heart beat is much greater than would be anticipated from the tracing given by the mercurial manometer. The highest pressure, which occurs while blood is passing from the heart into the aorta, is called the *systolic arterial pressure ;* the pressure at the end of diastole is the *diastolic pressure ;* and the range between these two extremes is known as the *pulse pressure.* In many animals the systolic pressure, as measured in the carotid artery, is under normal

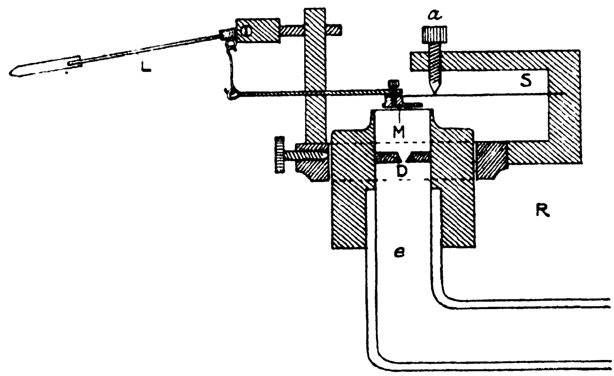

FIG. 399. Diagram to show the principle of the membrane manometer. M, the rubber membrane ; S, the spring ; e, connecting tube to artery ; D, damping diaphragm, L, the lever. (FRANK.)

conditions about 120 mm., while the diastolic pressure is only about 75 mm., so that the pulse pressure is about 45 mm. Hg. As we pass outwards towards the periphery the pulse pressure becomes less and less, until finally, in the capillaries and veins, there is usually no pulse wave perceptible.

THE VENOUS PRESSURE. In experimental animals the venous pressure is recorded by connecting a branch of the required vein with a manometer filled with salt solution or 1 per cent. citrate, and provided with a light recording float. It is important that there should be no valve in or near the branch selected. Venous pressures near the periphery are not definitely pulsatile, but in the great veins there are considerable pulsations, the venous pulse (p. 669) and these need special methods of recording.

ARTERIAL AND VENOUS BLOOD PRESSURE IN MAN

ARTERIAL PRESSURE. The determination of the systolic pressure in the arteries is easily carried out by the use of Riva-Rocci's † sphygmomanometer, of which there are many modifications. This apparatus (Fig. 400) consists of a leather or canvas cuff about 10 cm. wide, which can be buckled closely round the upper arm. Inside this band is a rubber bag of the same shape, which communicates by a rubber tube with a mercurial manometer or other pressure gauge, and by a side tube either with an air pump, or with an outlet valve. The band is buckled round the upper arm and the fingers of the observer are placed on the radial pulse. The bag is then distended with air so that it exercises a

* HAMILTON, BREWER AND BROTMAN *Amer. J. Physiol*, 1934, **107**, 427.
† RIVA-ROCCI, *Gazz. med. di. Torino*, 1896, **51**, 52; HILL and BARNARD, *B.M.J.*, 1897 (ii.), 904.

pressure on the arm, the pressure being indicated on the mercurial manometer. Air is forced in until the radial pulse disappears. By means of the outlet valve the air is then let slowly out of the bag until the radial pulse is just perceptible. The height of the

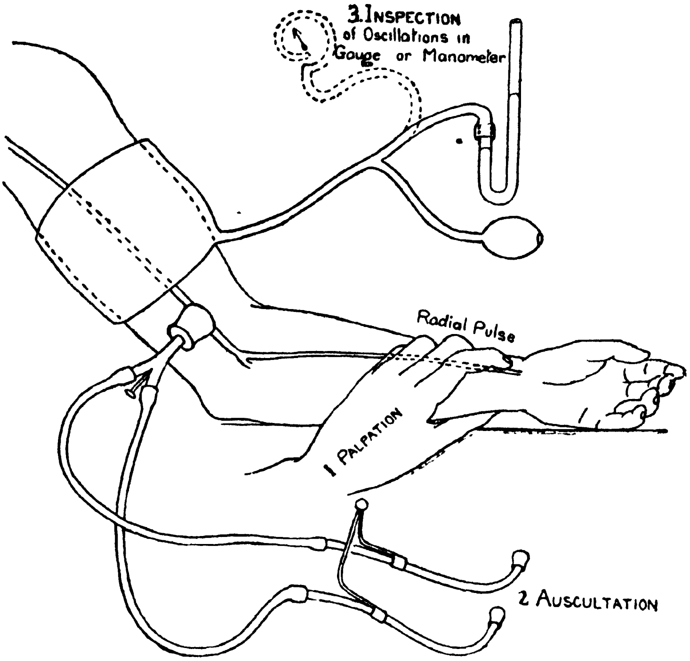

Fig. 400. Methods of using the Sphygmomanometer. (Outlet valve not shown) (Harris, "Experimental Physiology.")

mercurial manometer at this moment is equal to the systolic pressure in the main arterial trunk from which the brachial artery takes origin.

The principle of this method will be made clear by reference to the diagram (Fig. 401). If we imagine A as a segment of the brachial artery passing through the tissues which are surrounded by the rubber bag, we see that so long as the pressure in the interior of the artery is greater than that exerted by the tissues on the exterior, the artery will be patent and the pulse can pass through. If, however, the pressure in the tissues becomes greater than the maximum pressure inside the artery, the segment of artery will collapse (as in B), thus stopping the transmission of blood and of the pulse wave. If we exclude the elasticity of the tissues themselves, we may take the pressure in the bag as representing the pressure in the tissue fluids surrounding the artery, so that the pulse-obliterating pressure in the bag will correspond to the systolic pressure in the artery.*

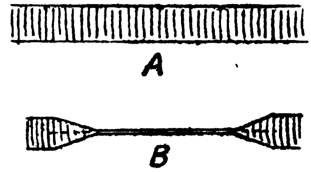

Fig. 401.

By a slight modification of the apparatus it is also possible to determine the diastolic pressure. For this purpose the rubber bag is connected with a gauge of small inertia, giving a true representation of the actual changes of pressure. When the pressure in the bag just exceeds the diastolic pressure, the artery will be completely collapsed in diastole, and will then dilate almost to the utmost with the systolic rise of pressure. If we are taking a record of the pressure changes in the bag in this way, the pulse waves as recorded by the manometer will slowly increase in size as the pressure in the bag is gradually raised from zero. At one point the waves rapidly reach a maximum, and the pressure in the bag at this point is taken as the diastolic pressure. As the pressure

* Mummery. *J. Physiol.*, 1905, 32, Pr. xxiii.

is still further raised, the excursions of the manometer diminish again, first slowly and then rapidly, and the point of rapid diminution corresponds to the systolic pressure. Above this point the manometer still shows small oscillations, due to the impact of the stump of the occluded artery on the upper border of the indiarubber bag.

THE AUSCULTATORY METHOD. The most convenient method of finding systolic and diastolic pressures in the artery is to apply the bell of a stethoscope over the brachial artery a little distance below the cuff of the Riva-Rocci apparatus. When the cuff is rapidly inflated to a point beyond the extinction of the pulse, and then gradually deflated, a series of sounds (" Korotkov sounds ") are heard, divided by Wiggers into five periods :

(1) As the pressure falls there is a sudden appearance of a clear sound, as the first flow of blood occurs through the compressed artery. This corresponds to the *systolic pressure.*
(2) The sound becomes more continuous, like a murmur.
(3) The sound becomes progressively louder.
(4) The sounds suddenly become muffled ; this is the *diastolic pressure.*
(5) The sounds disappear.

The arterial pressure varies over a wide range in normal subjects, and over a narrower range from time to time in any given healthy subject. Values often quoted are 120 mm. Hg. systolic and 75 mm. Hg. diastolic, but it is incorrect to accept these values as fixed standards of normality.* A survey of systolic blood pressures in 7,216 apparently normal persons between sixteen and forty years gave the following results (Alvarez †) :—

	Age.	
	18 years.	16–40 years.
Number of cases	1,216	6,000
Mean systolic pressure	130 mm. Hg.	128·9 mm. Hg.
Standard deviation	13·4 mm.	13·5 mm.

Unless we are to suppose that a proportion of these people were in an early stage of disease which at the time had produced no other effects, the figures mean that one-

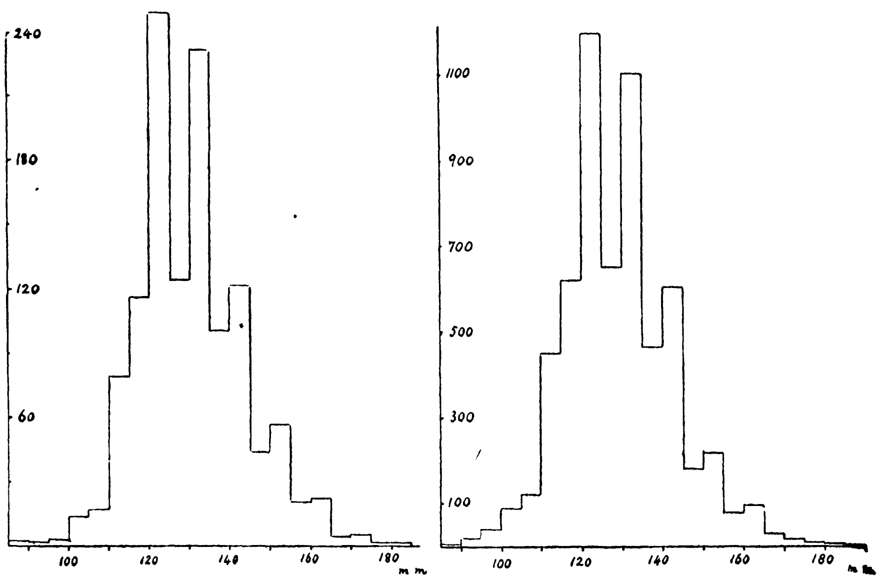

FIG. 402. Showing Frequency-distribution of the Normal Systolic Blood Pressure. (From Alvarez's tables.) Blood pressure as abscissa, number of persons as ordinate. On the left is shown the distribution of figures for 1,216 persons of 18 years of age. On the right figures for 6,000 persons from 16 to 40 years of age. (BURN. *Proc. Roy. Soc. Med.*, 1934, **28**, 15.)

* BURN. *Proc. Roy. Soc. Med.*, 1934, **28**, 15. TREADGOLD. *Lancet*, 8th April, 1933.

† ALVAREZ. *Arch. Int. Med.*, 1923, **32**, 17.

third of all males between the ages of sixteen and forty years of age have normal systolic pressures less than 116·5 mm. Hg. or greater than 143·5 mm. Hg., *i.e.* one-sixth will have pressures normally over 143·5 mm. Hg. One man in about every forty will have a normal pressure greater than 157 mm. Hg., or less than 103 mm. Hg. The figures actually ranged from 85 mm. to 180 mm., in a distribution shown in the form of a frequency diagram in Fig. 402.

VENOUS PRESSURE. The pressure may be approximately estimated in the superficial veins in man by altering the elevation of the part containing the vein so that the vein just collapses: the horizontal distance of this level from the heart, or from some agreed surface marking, such as the manubrio-sternal junction, is then measured. This method of course only gives minimal pressures, the pressure in the feet when standing, for instance, being greater than when lying with the leg in such a position that the veins just collapse.

In order to determine the venous pressure directly the simplest way is to pass a hollow needle into the vein, and connect it with a citrate manometer by a connecting tube entirely filled with fluid. The level of the citrate solution is then referred to that of the manubrio-sternal junction; records of variations can be taken by connecting the open limb of the manometer to a tambour.*

Another method depends on the use of a principle introduced by v. Recklinghausen.† One of the modifications of this method is by Krogh, Turner and Landis ‡ (Fig. 403).

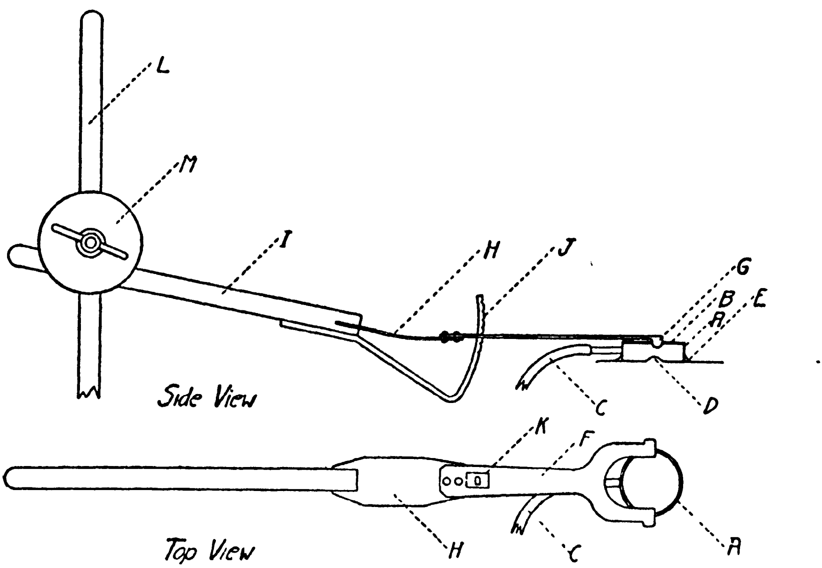

FIG. 403. Capsule for Measurement of Venous Pressures in Man. (KROGH, TURNER and LANDIS. *J. Clin. Inv.*, 1932, **11**, 357.)

A celluloid capsule A, with a celluloid top B, and a notch D on each side for the vein, is cemented to the skin over the desired vein, and held in position by a clamp having a steel spring H by which suitable pressure can be put on the capsule. A side tube C leads to a pressure bulb and water manometer, by which the air pressure inside the capsule can be altered to a known extent. The air pressure necessary to just collapse the vein is found, and is taken as equal to the venous pressure.

In the measurement of venous pressures it is customary to refer the hydrostatic level to that of the heart, but this is a purely arbitrary point and necessarily vague. It has in fact been shown that in the dead animal the reference point is caudal to the heart and is the same whether in the head-up or in the head-down position: in the living animal there are two reference points owing to the fact that the column of venous

* DOUPE, KRYNAUW and SNODGRASS. *J. Physiol.*, 1938, **92**, 383.

† v. RECKLINGHAUSEN. *Arch. f. exp. Path. Pharm.*, 1906, **55**, 463. HOOKER. *Amer. J. Physiol.*, 1916, **40**, 43.

‡ KROGH, TURNER and LANDIS. *J. Clin. Invest.*, 1932, **11**, 357.

blood is interrupted by the heart; one point for the head section is above the heart and one for the tail section is below the heart.* Owing to the uncertainty of the position of these reference points it is a common practice to measure the venous pressure levels from some definite anatomical point, *e.g.* the manubrio-sternal junction.

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ELEMENTARY HYDRODYNAMICS OF THE CIRCULATION

The blood flows through a branched closed system of tubes of elastic properties and of extremely variable diameters; moreover it is not a homogeneous liquid. Also, it is pumped intermittently into the arteries. The physical principles involved are consequently of great complexity and are for the most part as yet incapable of strict mathematical treatment. Nevertheless, certain elementary principles of hydrodynamics help towards a partial elucidation of the problems involved.

The cause of the pressure gradients in the different parts of the vascular system will be rendered clearer by a study of the flow of fluid through a tube of uniform bore Fig. 404) from a reservoir, the level in which is maintained constant, and which therefore exerts a constant hydrostatic pressure. It really amounts to the statement that water flows downhill. If the tube AG be connected with the reservoir R, fluid will flow out from G under the influence of hydrostatic pressure. The pressure on the fluid at each part of the tube can be measured by attaching at a series of points, *e.g.* at B, C, D, E, F—vertical tubes in which the fluid will rise to a height corresponding to the lateral pressure

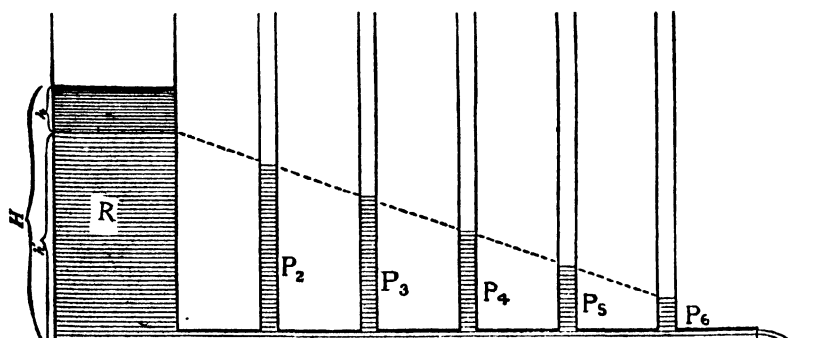

FIG. 404.

existing at these several points. If AG is of uniform bore, it will be found that the heights of the fluid in the tubes show a continuous descent, so that the line joining the tops of the fluid in the various tubes is a straight one. The movement of the fluid from D to E can be regarded as due to the difference of the pressure between D and E, *i.e.* $P_4—P_5$. It will be noticed in the diagram that the straight line joining the tops of the fluid does not strike the surface of the fluid in R, but falls a little below it. Of the total potential energy represented by the pressure H in R, the large portion h' is employed in overcoming the resistance to the flow in the tube AG, while the remainder h represents the force which gives to the fluid as it leaves the reservoir at A a certain velocity. The potential energy of the flowing liquid diminishes as it moves along, as is shown by the fall of lateral pressure, but the kinetic energy is constant throughout, in such a uniform tube. If the flow of fluid be diminished by partially clamping the end at G, or by increasing the length of FG, the rate of fall of the pressures will be diminished. This is

* CLARK, HOOKER and WEED. *Amer. J. Physiol.*, 1934, **109**, 166.

because in such a type of flow the velocity varies inversely as the frictional resistance, and *vice versâ*. A smaller portion (h) of the hydrostatic pressure is now used to impart velocity to the fluid, and a larger portion, h', to overcome resistance. The same effect will be produced either by raising the level of G or by lowering the level of the reservoir and so the pressure at A. This is because the velocity, and hence the frictional resistance, depends on the pressure difference.

The difference of pressure between any two points, *e.g.* between D and E, may be regarded as that pressure which is necessary to maintain a certain volume-flow of the fluid against the resistance offered by the friction of the fluid in contact with the walls of the tube. This friction, and therefore the resistance to the flow, can be increased by diminishing the diameter of the tube, when a larger difference of pressure will be necessary in order to maintain the same flow. This can be shown by introducing a resistance between D and E by partially clamping the tube at this point (Fig. 405). The uniform fall of pressures in the vertical tubes is at once abolished. Between A and D there is a continuous, but slower, fall, which is succeeded by a steep fall (P_4—P_5) between D and E, and this again by a gradual fall between E and G. Actually the volume of fluid flowing in unit time from a constant pressure source steadily along a straight tube

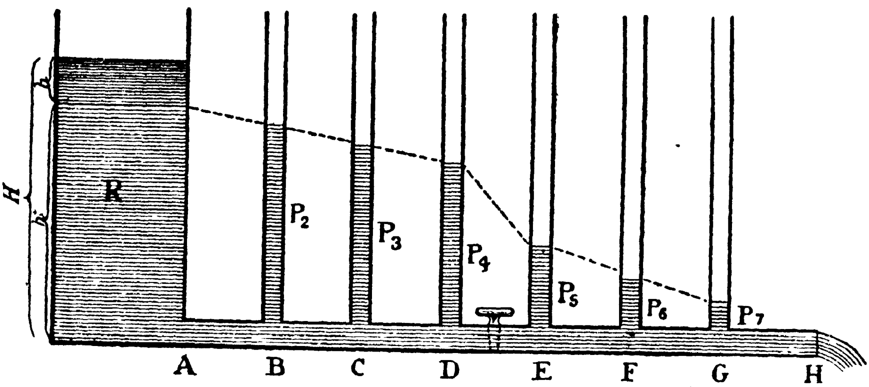

FIG. 405.

varies as the fourth power of the radius of the tube and as the pressure difference between its ends, and inversely as the length of the tube and the viscosity of the liquid (η). This is Poiseuille's formula : Vol. $= \dfrac{\pi P r^4}{8\eta l}$.

In the scheme shown, the reduction of the diameter of the tube at one point necessitates a greater difference in pressure between D and E than between the other points, in order to drive equal volumes of fluid through each section of the tube ; the total flow is also reduced for the reasons given above. The mean velocity of movement of the liquid in a tube is $\dfrac{\text{volume flow}}{\pi r^2}$ and hence, from Poiseuille's formula Vel $= \dfrac{Pr^2}{8\eta l}$. In any such system of tubes, therefore, through which fluid is flowing, the fall of pressure between any two points will be $= \dfrac{\text{Vel} \times 8\eta l}{r^2}$, *i.e.*, will be proportional to the product of the velocity and the resistance to the flow $\left(= \dfrac{8\eta l}{r^2}\right)$ between these two points. The velocity on the other hand, $\left(= \dfrac{Pr^2}{8\eta l}\right)$ will vary directly as the difference of pressures, and inversely as the resistance between the two points. These relations may be expressed by the simple formulæ

$$P \propto VR \quad \text{and} \quad V \propto \frac{P}{R}.$$

The scheme we have just considered is only capable of limited applications to the problems of the circulation. A nearer approximation could be got by studying the

problem, not of a flow from a constant pressure source, but that of a constant *volume* of fluid in unit time, as from a rotary pump, along the tube of uniform bore (Fig. 406). This would more nearly represent the conditions in the circulation, except for the fact that in the latter the flow is intermittent. Again we have a gradient of pressure, as shown by the vertical tubes A to F; the gradient will be steeper the narrower the horizontal tube and the greater the volume of fluid delivered in unit time by the pump; *i.e.* its steepness will depend on the energy needed to overcome the frictional resistance in the tube. At the free opening G, the fluid will have no lateral pressure, but will have a certain kinetic energy, dependent on its velocity, *i.e.* on the difference between the potential energy at the source and the frictional loss in the tube. If now an increase of resistance be put in at X, as by partly closing a tap, the effect will be of the same nature, but much more marked than with the former scheme, because now there can be no question of the resistance causing a diminution of the flow, which by definition, is constant. The effect will be a considerable rise of pressure in the near side and an equally pronounced fall on the far side of the resistance (Fig. 406).

Next, let us consider the effect, on the same schema, of altering the volume of fluid flowing through the horizontal tube. It will be found that the greater the volume

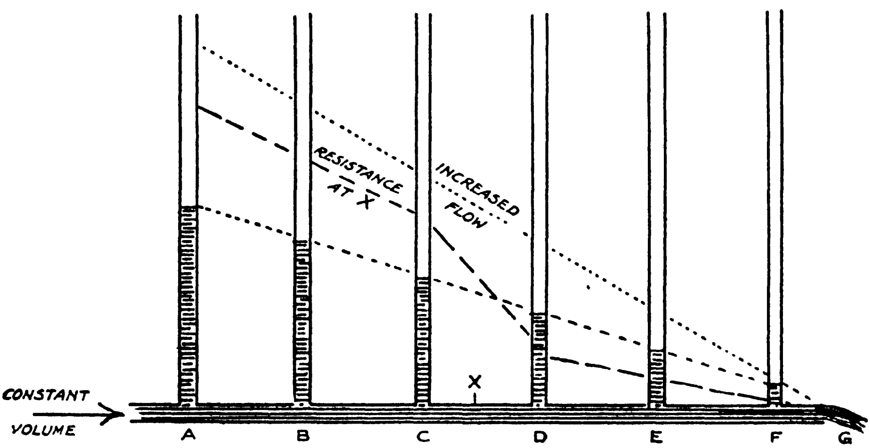

FIG. 406.

delivered in unit time the higher the lateral pressure near its entry will be, and the steeper the gradient (Fig. 406).

We have supposed in all these examples that the movement of the fluid is "stream lined." In this, the simplest type of fluid motion, the liquid moves in a straight line as concentric fluid tubes; the layer in contact with the walls of the tube is stationary, the next layer glides over it slowly, the next one over that, and so on until, at the axis of the tube, we have the maximal velocity. In such a case the resistance, which is due to the friction of the layers over one another, varies directly as the mean velocity. When, instead of being stream-lined the flow is whirling or turbulent, which happens above a certain critical velocity, even in straight tubes, the resistance varies approximately as the square of the mean velocity.* A formula used by engineers for such cases is $R = kSv^2$ where R is resistance, S is the surface area of the wall, and v the velocity. The higher the viscosity of the fluid, and the narrower the tube, the more readily does turbulent motion ensue, and hence the greater becomes the resistance.

We may now apply some of these principles to the vascular system. In the living vascular system, the largest difference of pressure exists between the arteries on the one side and the small veins on the other, a great fall occurring between the small arteries and the capillaries themselves. This distribution of pressure points to the chief gradient in the vascular system as being situated in the arterioles. The resistance presented by these vessels is due to their small calibre, since they are maintained in a state of tonic contraction. The total bed of the stream in the region of the arterioles, while greater than that of the arteries, is considerably less than that of the rich meshwork of capillaries, while the difference between the diameters of arterioles and dilated capillaries is not very

* WIEN-HARMS. *H'dbch. d. exp. Physik.*, 1931, IV. (1.), 293.

great. On this account the velocity of the blood in the arterioles is very much greater than that obtaining in the capillaries, and since friction, and therefore the resistance, probably varies as the square of the velocity, the resistance to the flow of blood through the arterioles must often be greater than that presented by the capillaries. When the capillaries constrict, however, they also add largely to the peripheral resistance. The large part taken by the arterioles in determining the difference of pressure between the arteries and veins is shown by the fact that this difference can be diminished to one-half by any means which causes a general dilatation of the arterioles, as for example, destruction of the vasomotor centre.

As regards the occurrence of turbulent motion, Muller * found that in an elastic tube only 7·5 mm. in diameter the flow began to be turbulent at a velocity of about 50 cm. per second. Hence, quite apart from turbulence due to branching, the flow in the larger arteries must be a turbulent one.

The mean arterial pressure, *i.e.* the average pressure within the arteries at any given time, is thus dependent on :

(1) The resistance to the outflow of blood from the arterial system, *i.e.* on the state of constriction of the arterioles and, to a smaller extent, of the capillaries.

(2) The output of the heart per unit of time.

The Effect of Branching. When a tube gives off a branch, the pressure exerted in the branch by a fluid *flowing along* the tube will depend upon the angle at which the branch leaves the main vessel. If, as at A, Fig. 407, the branch leaves at right angles, then the hydrostatic pressure in the branch is equal to the lateral pressure on the walls of the main vessel. If the branch inclines at an acute angle to the direction of flow, as

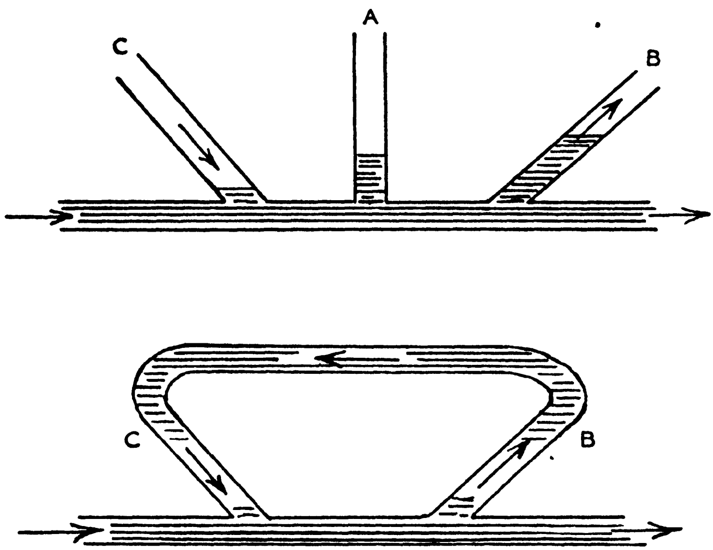

FIG. 407.

at B, then owing to the kinetic energy of the moving column of fluid, the pressure in B will be greater than the lateral pressure in the main stem, while if at an obtuse angle, as at C, it will be less. This is the principle of what is known as the *piezometer*, and it follows that if B and C are connected together, as shown in the lower diagram of Fig. 407, there will be a subsidiary (so-called piezometric) circulation of fluid along the side system in the direction indicated by the arrows. This is in fact what happens very extensively in many parts of the circulatory system, particularly in the smaller arterial anastomotic circulations, *e.g.* from arch of aorta to internal mammary artery, then *viâ* ascending aortic intercostal arteries to the descending aorta.†

Most of the arteries give off their branches at an acute angle, so that the end-on pressure in the branch is greater than it would otherwise be.

* *Ztschr. f. d. ges. Exp. Med.*, 1924, **39**, 210.

† SWINDLE, *Amer. J. Physiol.*, 1930, **93**, 588; BERRY and DALY, *Proc. Roy. Soc.*, 1931, **109B**, 333.

The Effect of Viscosity. For reasons already explained (p. 518), the apparent viscosity of blood, as measured by the rate at which it flows along a capillary tube under given conditions, is variable. Direct experiment shows that in passing through the capillaries of the vascular system, blood behaves as though it had about twice the viscosity of water, although glass capillary viscosimeters usually give a viscosity at least double this. The reason for the low viscosity in the circulation is due to the small diameter of the vessels in which the main resistance is met. In such narrow vessels the corpuscles tend to move like a rod in the middle of the stream, leaving the plasma at the periphery to encounter the frictional resistance of the wall.*

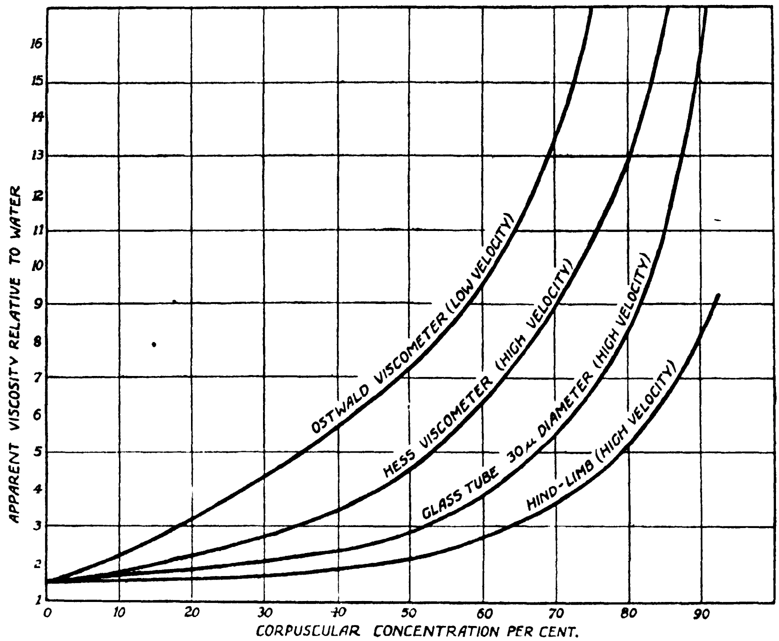

FIG. 408. The Influence of the Corpuscular Concentration (measured with a hæmatocrit) on the Apparent Viscosity of Blood.

Measurements were made on dog's blood, defibrinated, with four different types of viscometer: (1) the Ostwald (low velocity), (2) the Hess (high velocity), (3) a glass tube of very small diameter, and (4) the dog's hind limb. If the glass tube used had had an even smaller diameter, the apparent viscosities would have been still lower. (After WHITTAKER and WINTON, and L. E. BAYLISS.)

Increase in the corpuscular content increases the viscosity, *i.e.*, more pressure is required to maintain a given flow along a given capillary; in other words, if the corpuscular content is increased a given capillary tube will offer a greater resistance to the flow (Fig. 408).

THE EFFECT OF CAPACITY. So far, we have considered only the influence of changes of flow, pressure and resistance in a simple tube with a head of pressure at one end and a free outflow at the other. In the body, however, the vascular system is a closed circuit of branched elastic tubes presenting varying resistances to the flow of blood, and of varying diameters and distensibilities at different parts of their course. In this closed system is inserted a pump which drives the blood through the system. Since all the blood vessels are elastic and distensible, the capacity of the system must vary with the internal pressure to which the vessels are subjected. Moreover, the position of the different parts of the body must have an

* WHITTAKER and WINTON. *J. Physiol.*, 1933, 78, 339.

influence on the capacity of the system, since the dependent vessels will be distended, not only by the average pressure of the fluid throughout the system, but also by the hydrostatic pressure due to the weight of the column of fluid above them. The elasticity of the tubes is also a varying factor, and can be considerably altered by the contraction of the muscular coats of the vessels, or by pressure exerted on the vessels by the surrounding muscular and elastic structures.

Circulation Schema. It will simplify the discussion of the main factors of the circulation if, for the present, we neglect the variable factors and see what would take place in such a closed system of elastic tubes all situated on one horizontal plane. Such a system is represented in the diagram (Fig. 409), and a working model of it in Fig. 410.

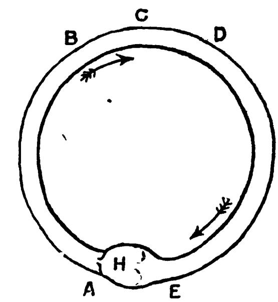

Fig. 409.

The heart H is interpolated at one part of the circuit, while the free flow of the fluid from B to D is impeded by the presence of a peripheral resistance at C. Such a system would have a definite capacity at zero internal pressure, but a much greater amount of fluid might be forced into it under a positive pressure. We will assume that the pressure throughout the system when at rest is 10 mm. Hg., *i.e.* the elastic tubes are all slightly distended. If the heart H now begins to contract, it will pump fluid from E into A. The pressure in E will fall, while that in A will rise, the resistance at C preventing the free escape of fluid from B to D, and so causing the heart to pile up the fluid which it has taken from E into A.

If the properties of the tubes were uniform throughout the system, the rise of pressure in A would approximate very nearly to the fall of pressure

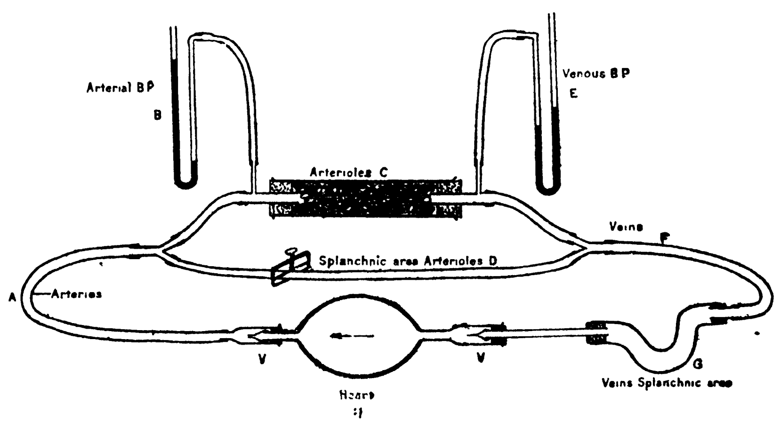

Fig. 410. Artificial Schema to Demonstrate the Main Features of the Circulation.

The heart is an enema syringe with valves at V and V. The artery is a thick-walled rubber tube. On the venous side is placed a length of wide thin-walled tubing, to represent the large thin-walled distensible veins. The arterioles and capillaries (peripheral resistance) are represented by wide glass tubes packed with sponges. By opening the clamp on the tube D ('splanchnic area arterioles') the peripheral resistance can be removed, and a free passage of fluid allowed from arterial to venous side.

in E. In the vascular system the veins are, however, much more easily distended or collapsed than the arteries (Figs. 326 and 327). The

vein is collapsed when there is no distending force in its interior, so that at zero pressure its capacity is nothing. The slightest rise of pressure causes a considerable increase in its capacity, and the capacity rises rapidly with increasing pressure up to about 20 mm. Hg. Whereas the artery needs a pressure of about 100 mm. Hg. to distend it, and continues to stretch, but more slowly, at higher pressures, the vein is distended almost to its maximum at about 10 mm. Hg. If, therefore, the tubes at E are made of thin-walled rubber, they will be considerably distended under a pressure of 10 mm. Hg., which has practically no influence on the thicker-walled arterial tube A.

A small amount of fluid taken from E would thus cause very little fall of pressure on this side. A considerable force will be necessary to send this fluid into the more resistant arterial tube, so that on pumping enough fluid from E to A to lower the pressure in E, say 5 mm., the pressure in A has to be raised to, say, 100 mm. Hg. in order to distend the arteries to such an extent that they will accommodate the extra fluid.

In such a system, when the heart contracts, it takes fluid from the veins to the arteries, and piles up the pressure on the arterial side until it is sufficient to cause fluid to flow through the peripheral resistance into the veins at the same rate as it is taken by the heart from the veins. This rise of pressure in the arteries may be many times greater than the fall of pressure in the veins. If more fluid is injected into the system, the whole system will be more distended and the *mean systemic pressure will rise.* Then, according to the rate of output by the heart, the arterial pressure may be less than, equal to, or greater than the pressure attained before the introduction of fluid. Supposing it to be the same as before, the increased amount of fluid must be accommodated somewhere, so that the venous pressure must be greater. In the same way, the withdrawal of a certain amount of fluid may lower the mean systemic pressure, and it would still be possible for the pump to maintain an arterial pressure equal to that produced when the mean systemic pressure was 10 mm. Hg., but to obtain this effect the rate of its output would have to be increased, and the veins would be more empty than they were previously. The maintenance of a constant arterial pressure with varying amount of fluid in the system can therefore be accomplished either by alterations in the output of the heart or by alterations in the peripheral resistance, and therefore in the ease with which the blood is allowed to escape from the arterial to the venous side.

Alterations of the capacity of the system will have the inverse effect to alterations of its contents. Thus diminution in the volume of veins, such as might be caused in the living body by pressure on the veins from without, will drive the fluid into other parts of the system and therefore raise the mean systemic pressure. This rise of pressure may be confined to the arteries by increased action of the heart, or it may be confined to the veins by diminished action of the heart or decreased constriction of the arterioles forming the peripheral resistance.

Similar change in capacity may be brought about by alterations in the hydrostatic pressure in the vessels. If, in the model illustrated (Fig. 410), we allow the thin-walled vein to hang over the edge of the table, the pressure of the column of fluid within it causes it to distend and to accommodate more fluid, and this increased capacity might be so great that the pressure in the section of the vein near the heart might fall so low that the heart would receive no blood. The whole arterial system might in this way drain under the influence of gravity into the large veins.

All the conditions in our artificial schema have their exact analogue in

the living body. The determination of the mean systemic pressure in the living body is difficult to carry out with accuracy. Direct measurement shows that it is not more than 10 mm. Hg. Under the influence of gravity, the pressure would be easily reduced to zero, or, in the upper parts of the body, even to a pressure below that of the air, but for the nervous reactive mechanism which we shall consider later on, and the whole of the blood would accumulate in the abdomen and lower parts of the body, and the circulation would come to a standstill.

Widespread or universal dilatation of the capillaries, as may occur under the influence of histamine, or in surgical shock, may so increase the total capacity of the vascular system that the blood remains in the capillaries, and an ever decreasing amount of blood is available to pass on into the heart. The heart thus has a diminished output, and is unable to maintain the normal arterial pressure in the arterial system. The *mean pressure* may be altered in the vascular system in the following ways :

(1) Alteration of capacity of the total system, either by contraction of the walls of the vessels or by pressure on them from without.

(2) Alteration of the total volume of the circulating fluid.

The *distribution of pressure*, *i.e.* the relative pressure in the arteries and veins, will be determined by

(3) Alteration in the output of the heart. This may be augmented by greater amplitude of ventricular contraction increasing the output per beat, or by greater frequency of beat. Generally both means are employed.

(4) Alteration in the peripheral resistance and therefore in the ease with which the blood can escape from arterial to venous side.

In any change, either in arterial or venous pressure, at least two of these factors are involved. Every constriction of arterioles causes, not only an increase in the peripheral resistance, but also a diminished capacity of the whole system, so that the arterial pressure is raised at the same time as the mean systemic pressure. Nearly always such a change will involve as its immediate consequence some corresponding alteration in the heart beat, so that at least three factors will usually co-operate in the production of the rise or fall of blood pressure.

The Dependence of Arterial Pressure on Output of Heart. Arterial pressure is a resultant of the two effects :

(*a*) The rate at which blood enters the arterial system from the heart ;

(*b*) The rate at which blood leaves the arterial system through the peripheral resistance.*

It is evident that the arterial pressure will be altered by varying either of the two factors—output of the heart or peripheral resistance. The cardiac output will depend on the stroke volume of the heart and on the pulse rate. The filling of the heart at the beginning of each beat is in its turn dependent on the pressure in the great veins. If the heart is beating with optimum rate and force, it will keep the veins nearest the heart practically empty, and it is not possible for it to obtain more blood to put into the arterial side, however frequently it may beat. Increased frequency of heart beat would not, therefore, necessarily increase the total output of the heart. There will be an optimum frequency of the heart beat which will depend on the state of filling of the great veins. The fuller these are, the more the total output can be augmented by an increase of the heart rate. On the other hand, with the heart beating at its optimum rate and with effective systole, slowing the heart rate will diminish the output and therefore the arterial pressure.

* For hydrodynamical treatment *v.* BROEMSER and RANKE. *Z. f. Biol.*, 1930, **90**, 467; WEZLER. *Ibid.*, 1938, **98**, 438.

BLOOD FLOW

The Conversion of an Intermittent into a Constant Flow. By the time it has arrived in the veins, the flow of blood has been converted from a pulsatory into a continuous flow. This change is connected with the elastic nature of the arterial walls and will be more easily understood by a simple illustration. If a pump be connected with a rigid tube, every time that a certain amount is forced into the beginning of the tube an exactly equal quantity will be forced out at the other end. Increasing the peripheral resistance by partial closure of the end of the tube will not affect the intermittent character of the flow, but will merely increase the force necessary to expel the fluid. If, instead of a rigid tube, we employ a wide elastic tube, and the end be left open so that no resistance is offered to the outflow, the outflow will, as with a rigid tube, be intermittent. But now, if the end of the elastic tube be narrowed so as to increase the resistance to the outflow, there will be a marked difference. Each stroke of the pump forces the same amount of fluid into the tube as before, but owing to the peripheral resistance this does not now all escape at once; instead, part of the force of the pump is spent in distending the walls of the tube, and part of the fluid that was forced in remains for a time in the tube. The distended tube tends to empty itself and force out the fluid which overdistends it before the next stroke of the pump occurs. So now the outflow may be divided into two parts, one part which is forced out by the immediate effect of the stroke of the pump, and another part which is forced out by the elastic recoil of the tube between the strokes. If the strokes be rapidly repeated before the tube has time to empty itself thoroughly, it will get more and more distended. Greater distension means stronger elastic reaction, and therefore a larger outflow of the fluid between the beats. This distension goes on increasing till the fluid forced out between the strokes by the elastic reaction of the wall of the tube is exactly equal to that entering at each stroke, and the flow thus becomes continuous.

The same thing occurs in the living body. A man's heart at each beat forces about 60 c.c. of blood into the already distended aorta. The largest arteries, such as the aortic arch, innominate, and subclavian arteries are highly elastic and distensible, and so the expelled blood is readily accommodated in them; this wave of distension is transmitted with gradually decreasing force along the arteries and is what we feel as the pulse. After each heart beat the arteries tend to return to their original size, and drive the blood onwards. By the time the blood has reached the veins, all trace of the heart beat has disappeared, and the pressure has fallen to a few millimetres of mercury.

VELOCITY OF THE BLOOD

The velocity of the blood as a whole will vary directly as the rate of the cardiac output, but, apart from changes in this, the speed of movement of the blood in different parts of the circulation will always vary greatly, for reasons already explained.

We may consider (1) the circulation time, *i.e.*, the time taken for blood to traverse a part or the whole of the circulation; (2) the linear velocity of the blood as it passes a particular spot, and such velocity may be considered either as a mean velocity or as a velocity fluctuating from moment to moment; (3) the volume of blood flowing through a particular part of the circulatory system.

(1) **Circulation Time.** Measurements of the circulation time are made by introducing into the blood stream some easily recognised substance at a given point, and finding how long it takes for that substance to appear at any other given point in the circulatory system. If the tell-tale substance is injected into a vein on one side of the body, and the time taken for it to appear in the blood of the same vein further up-stream, or in the corresponding vein on the opposite side of the body, then we get the *total circulation time*; if the transit is from vein to lungs we get a measure of the time for the pulmonary circuit; if from vein to capillaries we get the time for flow from vein through pulmonary circuit, left heart and arteries to capillaries, *i.e.* total circulation time less the time for venous return, etc.

Substances which can be used for injection are: (1) Potassium ferrocyanide, in animals; (2) radioactive material, detected at various points, without drawing blood, by the use of an ionisation chamber; (3) the dye fluorescin, for total time; (4) acetone or ether, detectable in the breath, for pulmonary circuit time; (5) histamine (0·001 mg. phosphate per kg.), which causes flushing of capillaries of the face, or decholin (sodium dehydrocholate) 1 g., which gives a bitter taste when it reaches the tongue, for the vein-heart-capillary time.

Typical times found are:

Total circulation time (vein to vein) .	12 to 28 secs. (av. 25)
Arm vein to face (histamine) . . .	24 secs. (decholin 11 secs.)
Arm to artery (radio-active method) .	14 to 24 secs.
Vein to lungs (ether or acetone) . .	6 secs. (radio, 11 secs.)
Arm to heart (radio-active method) .	2 to 14 secs.

It will be evident that the time measured in all these methods is the shortest time taken by any of the blood to traverse the given part of the circulation, and hence the figures are variable unless exactly the same method is used. For different animals the circulation times are shorter the smaller the animal, as would be expected. The number of heart beats per circulation time, is, however, fairly constant for all species, viz., about thirty beats per total circulation time.

Any factor which increases the cardiac output will shorten the circulation time. Hence it is reduced in exercise, after adrenaline and in such diseases as hyperthyroidism, where the total metabolism is raised, or anæmia, in which the blood volume is increased and its viscosity diminished. It is reduced in myxœdema, because the basal metabolism is reduced, in polycythæmia, because the viscosity of the blood is increased, and in decompensated disorders of the heart.

(2) **The Velocity of Flow at Different Points.** It is evident that an increased output of the heart in unit time will speed up the rate of flow of blood in all parts of the circulation. At any given rate of output, however, the velocity of the blood varies as it passes along the vessels. If a liquid be flowing steadily along a tube of unequal bore, as represented in Fig. 411,

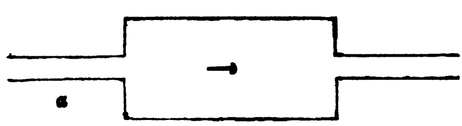

FIG. 411.

since the amount of fluid passing *a* during a given interval of time must be equal to the amount passing *b*—where the bed of the stream is wider—the velocity of the flow must be smaller at *b* than at *a*.

It is obvious that the velocity of flow at any two points in the same

system will vary inversely as the cross-sectional areas of the whole bed at those points. Thus in a closed circuit (Fig. 409) with a steady flow from the arterial to the venous side, the amount of fluid passing A during a minute must be exactly equal to the amount passing through the peripheral resistance C. The total area at C is, under average conditions in the body, probably one thousand times that of the aorta at A, and we should expect therefore a proportionate slowing of the blood stream. As a matter of fact, while the mean velocity of the blood in the aorta of a large animal may be taken as about half a metre per second (varying at different parts of the cardiac cycle from 0·1 to 1·0 metre per second), the velocity of the blood in the capillaries is about half a millimetre per second. Moreover, since the total cross section of the big veins near the heart under a normal distending pressure is about twice that of the first part of the aorta, the velocity of the blood in the great veins is only about half of that found in the aorta.

The foregoing discussion must not be applied directly to the changes in the velocity in any part following on *local* alterations in the resistance. In this case the local changes are insufficient to affect the general arterial blood pressure, and the result of diminution of peripheral resistance is to furnish a short cut from the arterial to the venous side for a portion only of the total output of the heart. Thus, dilatation of the vessels of the submaxillary gland, while not altering the general blood pressure, causes the blood flow through the gland to be increased six to eight times; and the peripheral resistance in the gland may be so far diminished that the blood passes into the veins without losing the pulsatile force imparted to it by each heart beat. The pressures and velocities in the *local* arterioles, capillaries and veins are all increased by this local vaso-dilatation. Conversely, constriction of the arterioles of any given part will diminish the velocity of the blood through this part and also the pressure in its capillaries.

The larger the area affected by the change in the peripheral resistance, the more difficult it is to predict *a priori* what will be the result on the velocity of the blood and on the circulation as a whole, or in the parts specially affected. In the living body a widespread dilatation of the arterioles increases the total capacity of the system. The arterial relaxation, therefore, not only gives rise to an easier outflow from arteries to veins, but may also cause a diminished filling of the veins, and therefore of the heart during diastole. The heart output is therefore also lessened, so that a final result of a dilatation of the arterioles may be a diminished, instead of an increased, velocity throughout the system.

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METHODS OF MEASURING THE VELOCITY OF THE BLOOD

If we can measure the average volume of blood passing along an artery per minute, we can calculate the mean speed of its movement. In experimental animals, the mean flow can be measured by Ludwig's *Stromuhr* (Fig. 412). This instrument consists of two bulbs of equal size, *e* and *e′*, communicating with one another above; their lower ends are fixed in the disc *c*, which is pierced by two openings serving to connect the lower orifices of the bulbs with the tubes cemented into the lower disc *b* over which *c* can rotate. The animal is first given an intravenous dose of heparin to render the blood incoagulable.

The tube *a* is inserted into the central end, and *a′* into the peripheral cut end of the artery. The tube *e* has been filled with oil and *e′* with blood. Blood flows into *e* and

drives the contained oil over into e', the contents of e being meanwhile forced on into the peripheral end of the artery. When blood has completely filled the bulb e, the two bulbs are reversed. Knowing the capacity of the bulbs and the number of times it has been necessary to reverse them in the course, say, of one minute, we know also the amount of blood which has passed through the artery under experiment, and, by calculation, its mean velocity.

This, however, does not give a graphic record, nor any information of the rapid changes occurring in the velocity of the blood at each heart beat. For this purpose we must have recourse to some such instrument as the *differential Stromuhr* used by Fleisch.* This is based on an application of Venturi tubes. If a current of blood, rendered incoagulable by a suitable method, be directed along the tube AB (or CB) possessing two vertical side tubes (Fig. 413), the pressure in the first branch will be greater than that in the second, since when the flow is from A to B, the momentum of the moving mass of blood is added to the lateral pressure of the fluid · if from C to B, there is still a difference, though a smaller one, due to the lateral pressure gradient along the tube. In recording the variations in blood flow, *e.g.* in an artery, the apparatus is inserted in the artery, so that blood enters it at A and leaves it at B to continue on its course along the vessel.

The difference of pressure will be greater if the flow accelerates, and will disappear if it ceases. It can be recorded by means of a membrane manometer, one surface of which is in connection with one branch and the other with the other branch, as shown in Fig. 413. The instrument must, of course, be calibrated empirically if the actual velocities are required. Fig. 414 gives a tracing taken with such an instrument, and illustrates the increase of blood flow which results when the splanchnic area is perfused, at constant pressure from a pump, with blood containing an increased amount of carbon dioxide. The velocity in the artery shows regular fluctuations with each stroke of the pump, as it would with the heart beat, an increase with each systole, and a decrease with each diastole (the tracing is on too slow a surface to show these clearly); there are also slower fluctuations in the form of waves (six are seen in the tracing) which are due to rhythmic changes in the vascular tone.

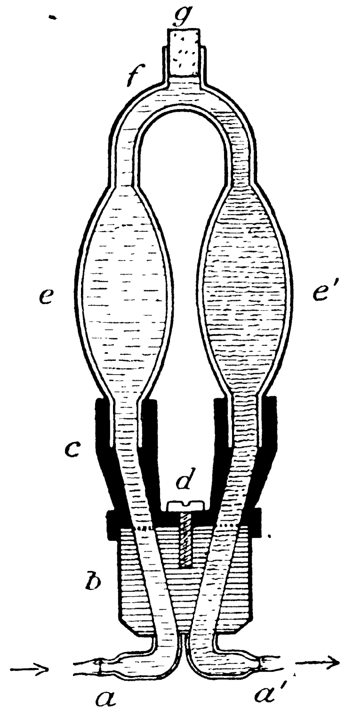

FIG. 412. Diagram of Ludwig's '*Stromuhr*' (From SHARPEY-SCHAFER'S' "Experimental Physiology." Longmans, Green.)

Another form of stromuhr is the *thermostromuhr* of Rein.† The principle is that a spot on the vessel is heated at a known rate (by a high-frequency current) and the rise of temperature is then recorded a short way downstream by a thermoelectric couple. The rise varies inversely as the velocity of flow. A method based on an electromagnetic principle has also been described.‡

The velocity of the blood in the capillaries can be measured by direct observation of the capillaries under the microscope, and noting the time it takes for a blood corpuscle to move from one edge of the field to the other.

During systole the velocity of the blood in any part of the arterial system is greater than during diastole; thus in the carotid artery the velocity during systole may be as great as 500 mm./sec., and during diastole only

* FLEISCH. *Pflüger's Arch.*, 1920, **178**, 31.

† REIN. *Z. f. Biol.*, 1929, **89**, 195. BARCROFT and LONGBRIDGE. *J. Physiol.*, 1938, **93**, 382.

‡ KOLIN. *Amer. J. Physiol.*, 1937, **122**, 197.

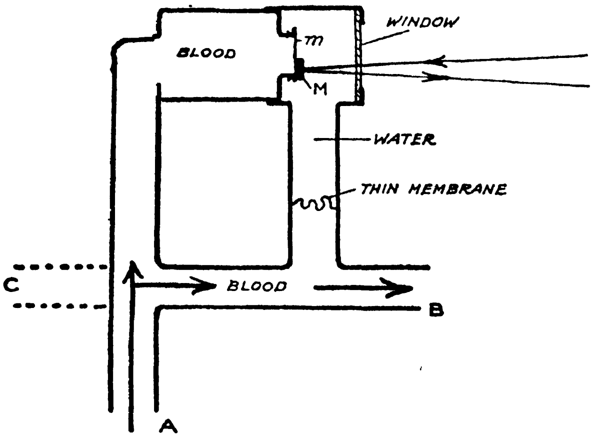

FIG. 413. Principle of the Fleisch Differential Stromuhr The difference in pressure in the two branches is communicated to the thin membrane manometer, *m*, bearing a mirror M. The outer chamber is filled with water, so that light passing through the window can be reflected from the mirror, and on to a photographic surface, whereby the pressure differences can be graphically recorded. The thin *lax* membrane separates the water from the blood, but does not interfere with the pressure transmission.

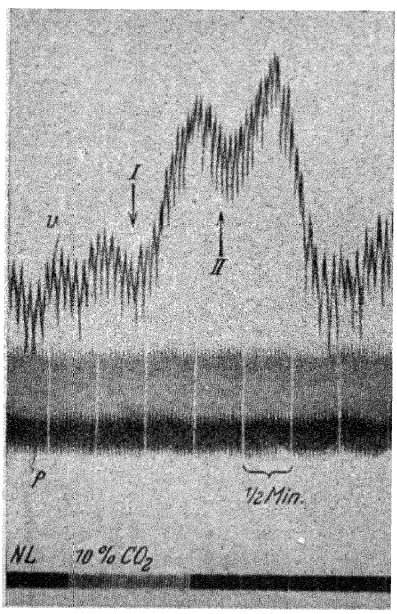

FIG. 414. Record of Velocity of Blood flowing along the Superior Mesenteric Artery of a Cat. The artery was perfused with blood from a pump, the perfusing pressure being kept constant, and the blood being arterialised in a separate pair of lungs. Upper tracing = blood velocity : the sudden downward strokes, about fourteen per minute at the commencement, are produced by a device which shows when each 1 c.c. of blood has passed through. They get closer together at the arrow II., and the whole tracing is moved upwards, both of which events show that the blood is flowing more rapidly. Second tracing : P = perfusion pressure, and time tracing in half minutes. Lower tracing shows the time during which the lungs of the perfusion circuit were supplied with a mixture containing 10 per cent. CO_2. (FLEISCH, *et al.*, *Pflüger's Arch.*, 1932, **230**, 814.)

two-thirds as great at the most. The fluctuations in velocity attending each heart beat are parallel to the fluctuations of pressure which we know as the pulse.

(3) **The Volume Flow.** The volume of blood which flows through any portion of the circulatory system in a given time bears no close relation to the actual speed of movement of the blood along the vessels concerned. For whereas the volume of blood transmitted at constant arterial pressure varies directly as the fourth power of the diameter of the small arteries that supply the part, and inversely as the resistance of the total circuit, the speed of movement at any given point under a constant head of pressure will vary inversely as the total sectional area of the local bed, at that point. It usually follows, therefore, that any dilatation of the arterioles supplying an organ leads to an increase in the velocity of movement of the blood, as well as to an increased input of blood to the organ.

The flow may be measured directly by actual collection of the blood, rendered incoagulable by heparin, as it emerges from a vein; the time required to collect a given volume is measured and the blood re-injected into the circulation. Or we may measure the blood entering by an artery, by inserting a stromuhr of some pattern on its course.

Another method is by the use of the plethysmograph (p. 674), by which the volume of an organ is recorded. If an organ is placed in a plethysmograph, and the outlet of blood by the veins be then obstructed for a definite *short* time, the organ will swell by an amount that can be read off from the record, so giving the amount of blood that entered it during the period of occlusion.

The flow of blood through the hand or foot in man can be arrived at by a method devised by Neil Stewart. The hand is immersed in a calorimeter containing a known amount of water, and the rise of temperature of the water in a given time is measured. Then, assuming that the heat given out is that carried to the hand by the warm blood flowing to it, the flow of blood can be calculated. Although only approximate, the method is capable of showing variations in the rate of flow through the hand with considerable delicacy. A usual figure is about 13 g./min./100 g. hand at 17° C.

The volume of blood flowing through the various organs shows wide variation, depending chiefly on the intensity of metabolism of the organ, and so on its oxygen requirement. If the organ becomes more active the blood supply to it is increased by dilatation of the arterioles, or capillaries, or both.

The following figures give approximate rates of flow per 100 g. organ per minute:

Thyroid	560 c.c.
Kidney	150 c.c.
Liver (1/3 arterial, 2/3 portal) . .	150 c.c.
Heart (coronary circulation) . .	100 c.c.
Brain	130 c.c.
Intestine	70 c.c.

Of the total output of the heart it is probable that in states of muscular rest more than half goes to supply the head, upper extremities and liver.*

* Levy and Blalock. *Amer. J. Physiol.*, 1937, 118, 368.

THE ARTERIAL PULSE

›lic rise of pressure at each heart beat causes an expansion, spoken of as the pulse, which can be felt in any superficially situated artery, such as the radial. As we have seen, the amplitude of the pulse decreases as we go farther away from the heart.

PALPATION OF THE PULSE. From time immemorial the physician has sought by feeling the pulse to come to some idea as to the condition of the heart. A number of different qualities have therefore been distinguished, *e.g.* the *rate* may be rapid or slow, the size or *amplitude* large or small, while the *hardness* of the pulse is determined chiefly by the blood pressure.

If the pulse is compressible it is spoken of as *soft*; if it can only be obliterated with difficulty it is *hard*. Certain combinations of these qualities are also described. Thus a large and hard pulse is spoken of as *strong*, a *weak* pulse being both small and soft. If the rhythm of the heart beat is irregular the pulse is also *irregular*. An *intermittent* pulse is one in which one heart beat is dropped occasionally, *e.g.* once in every four or eight beats, and may be due to the interposition of a ventricular contraction which is too weak to propel the pulse so far as the radial artery, or to the occurrence of a premature contraction, followed by a compensatory pause.

A distinctive pulse is that known as the '*water-hammer*' pulse, which is observed in cases where the aortic valves are diseased so as to allow of regurgitation. The systolic rise of pressure in the arterial system is followed by an extremely rapid fall. Under such conditions, if the arm be held above the head, the pulse in the arteries of the wrist is felt as a smart blow.

Cause of the Pulse Wave. If the arterial system were perfectly rigid, the increased pressure at each ventricular systole would occur practically simultaneously at every point. The arteries are, however, elastic and distensible, so that the first effect of the flow of blood into the aorta is to distend the section of the aorta nearest to the heart. The shock due to this suddenly increased tension is transmitted from segment to segment of the arteries in the form of a wave, at the velocity of about seven metres per second.

It is important not to confuse the velocity of the pulse wave with that

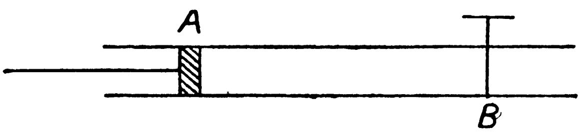

FIG. 415.

of the blood flow; the latter is never greater than 0·5 metre per second, and is very much less than this in the smaller arteries. Perhaps the difference between the two quantities may be made clearer by analogy. When a railway engine shunts a row of slowly moving trucks, a jolt is very quickly passed on from truck to truck until the end van is reached, though in that short time the train as a whole has moved a very short fraction of its length, by the constant push of the engine. In the arteries, this continuous pressure is furnished by the elastic reaction of the arterial wall, and we see how the impact of the blood may travel quickly as a wave of increased pressure, while the blood itself is moving slowly along, impelled by the reaction of the arterial wall.

In a rigid tube AB (Fig. 415) provided with a piston at the end A, and filled with fluid, an inward movement of the piston at A will cause a simul-

taneous outflow of fluid at the end B. If the end B is closed, the piston at A cannot be moved at all. Pressure applied to the piston will raise the pressure simultaneously at all points in the tube AB. The increased pressure applied at A is therefore transmitted with practically no loss of time to all parts of the tube AB. The pulsation will be retarded if we have a tube whose wall is distensible and elastic. If we imagine (Fig. 416) an elastic tube BC filled completely with water and connected at B to a rigid tube, which is provided with a piston, the first effect of a rapid movement of fluid driven in by the piston will be a rise of pressure at the point immediately in front of the piston, viz. at *a*. The wall being distensible, and pressure being propagated

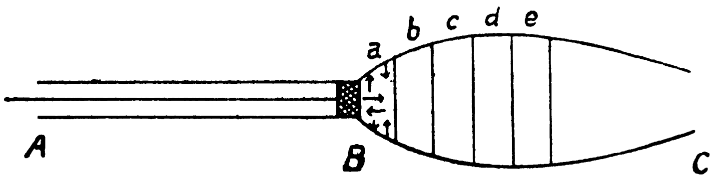

FIG. 416.

along the fluid in every direction, the rise of pressure at *a* will be spent partly on the particles of fluid in front of it, viz. at *b*, but also on the walls of the tube, so that this is stretched and the cross section of the tube enlarged. The distended segment at *a* will then exert a pressure on the contained fluid, driving this backwards and forwards. The fluid on its side towards the piston will tend to come to a stop, while that towards the distal end of the tube will be accelerated. The distended wall therefore returns to its original diameter, and the next segment at *b* is stretched in its turn, so that a wave of increased pressure is propagated along the tube in the direction of the arrow.

The velocity with which this wave is propagated depends on the density of the fluid, *i.e.* its inertia, and on the resistance of the walls of the tube to distension, *i.e.* on the rapidity of its recovery. The velocity of propagation of the wave of increased pressure, or the wave of expansion of the artery, is expressed by the following formula :*

$$v = \frac{3\ 57}{\sqrt{E}}$$

where v = velocity in m./sec., and E = per cent. increase in volume per mm. Hg increase of pressure.

The elastic coefficient of the arterial wall becomes larger as the arterial wall is distended. The velocity of propagation of a wave of pressure in the arterial system will thus be greater when the arterial pressure is high than when it is low.

If the end C of the tube is closed, the wave of positive pressure on arriving at C will be reflected back as a positive reflected wave. If a tracing be taken of the oscillations or variations of pressure in the tube, two waves at least are seen, one of which is the primary wave due to the movement of fluid caused by the piston; the other is the secondary wave reflected back from the periphery. That the secondary wave is a reflected one is shown by the fact that the nearer to the closed end the pulse is recorded, the nearer is the secondary to the primary wave, as is seen in Fig. 417.

If the tube BC be widely opened at C, a reflected wave is also observed, but this time the wave is one of reduced pressure. The production of this wave is dependent on the momentum of the moving column of fluid. If in the tube *ab*, with a tap at *c* and a manometer *m* (Fig. 418), the current of fluid be suddenly checked by turning the tap *c*, the column in front of the tap, having a certain momentum, will tend to go on moving and therefore produce a suction or 'negative pressure' behind it. When a wave of pressure arrives at the open end of a tube, there is a sudden drop in the resistance to the

* BRAMWELL and HILL. *Proc. R. S.*, 1922, 93B, 298.

output, and the momentum of the mass of fluid which is thrown out causes a similar suction or 'negative pressure,' which travels back the whole length of the tube. If the end of the tube is only partially closed, every primary positive wave will be transformed into a reflected one which is partly positive and partly negative. Since both

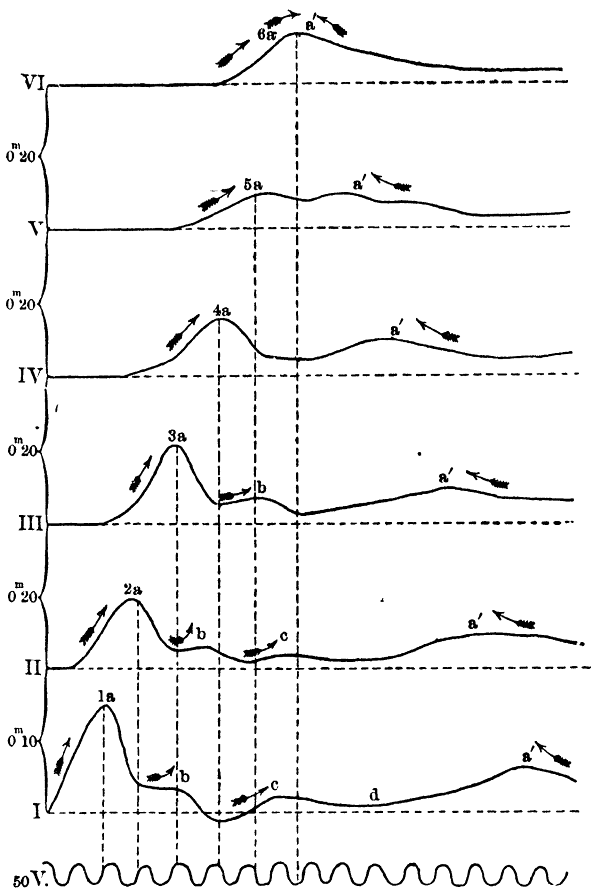

FIG. 417. Pulse Curves described by a series of levers placed at intervals of 20 cm. from each other along an elastic tube, into which fluid is forced by a pump. The pulse wave is travelling from left to right; a = primary and b, c = secondary pulse waves. The distances between vertical lines indicate the time taken up by the wave in passing along 20 cm. of the tubing. The waves (a') are waves *reflected* from the closed distal end of the tubing; this is indicated by the direction of the arrows. It will be observed that in the more distant lever (VI) the reflected wave, having but a slight distance to travel, becomes fused with the primary wave, so that the rise of pressure in VI is actually greater than that in V. (From FOSTER, after MAREY.)

these reflected waves travel through the tube with the same velocity and will mutually interfere, the result may be either a positive or a negative wave or nothing at all, according to the degree of constriction.

In a branching system of tubes, such as the arterial system, reflection of waves must take place at every division. All the conditions for the origination and interference of such waves are present in the arterial system. It is impossible, however, to say *a priori* whether any reflected wave will form a marked feature on the pulse tracing. It is possible that the multitudinous reflections which must occur in every part of the arterial system may interfere with one another to such an extent that they mutually annul each other.

The identity of any secondary wave in the pulse tracing must therefore be determined by exact measurement and experiment.

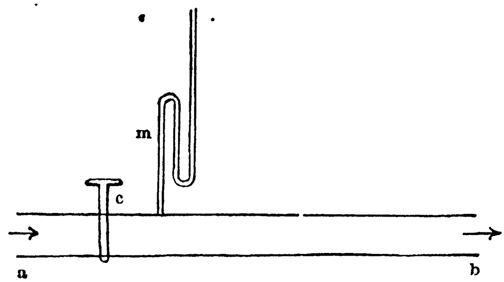

FIG. 418.

Pulse Tracings. To study the pulse it is necessary to obtain a graphic record of the expansion of the arteries or of the changes in pressure which produce this expansion. The former is the more accurate because, owing to the fact that the extensibility of an artery diminishes with rising pressure, a given increment of pressure causes a greater expansion at the commencement of the rise than towards its peak. For accurate records, a manometer

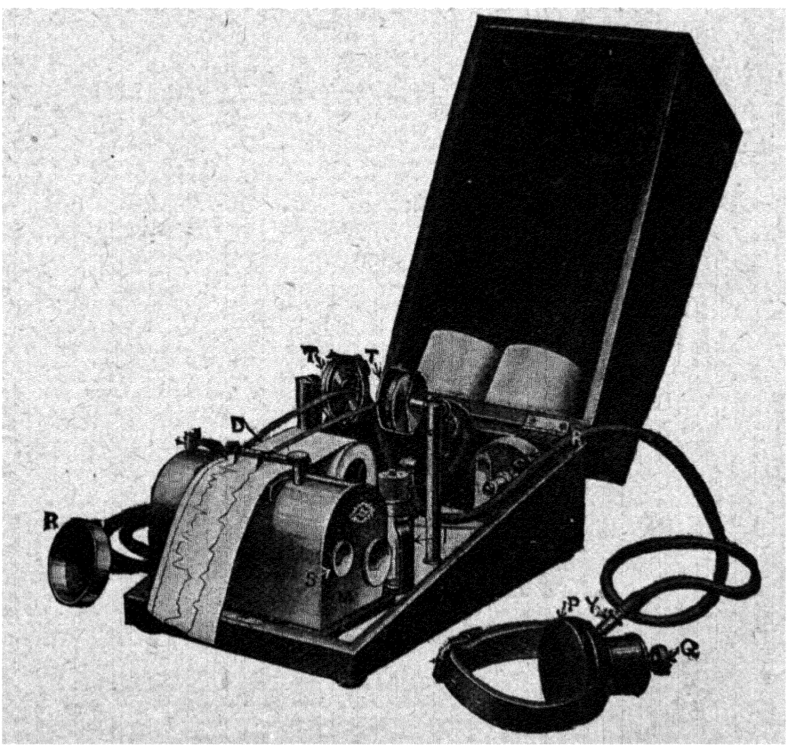

FIG. 419. The Polygraph. The record is made on the paper, wound out by clockwork from a roll, by the ink pens D. These are attached to levers of the tambours T-T, which are actuated by air-transmission from the receivers. P is the glycerin pelotte which can be strapped round the wrist; the pulsations of the radial artery cause movements of the air along the tube leading to the tambours, and so records the pulse tracing of the artery. R, an open capsule, can be used for application over the jugular vein, in recording the venous pulse. (CAMBRIDGE INSTRUMENT CO. LTD.)

which has very little inertia, such as Wiggers' (p. 579), must be used, or the expansion of the artery may be recorded photographically. The expansion of the artery can be approximately registered by means of a lever, which may be made to rest upon an artery, *e.g.* the radial, and the movements of which are recorded on an appropriate surface. Such an instrument is called a *sphygmograph.*

Alternatively, one of the various forms of *polygraph,* depending on air transmission by tambours, may be used. They have the advantage that records from two or three sources, *e.g.* from two different arteries, or from artery and vein, or cardiac impulse, &c. may be made simultaneously (Fig. 419).

In all such instruments the moving parts have considerable inertia, so that the curve they give is always deformed. This fact must be borne in mind when comparing the pulse curves obtained by means of a sphygmograph or polygraph with those given by the more perfect forms with optical methods of recording If an open cup be firmly pressed to the skin over an artery, such as the carotid or subclavian, and be connected by a tube with a 'segment capsule,' the excursions of which are recorded photographically, curves may be obtained reproducing in all particulars those given by optical manometers (Fig. 420).

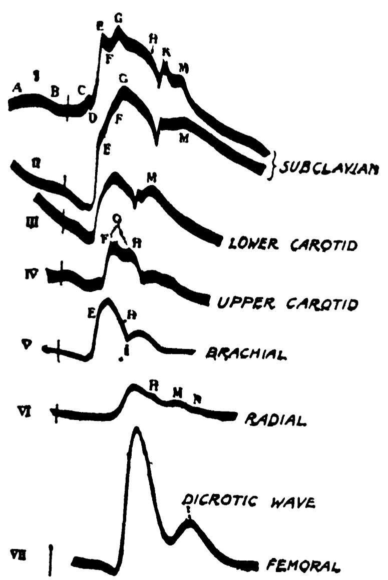

FIG. 420. Pulse Tracings from Arteries, taken by Air Capsules and Optical Recording. (WIGGERS.)

The pulse wave undergoes considerable modification in shape and time relations as it passes along the arterial system. The time-pressure, or time-volume curves taken in the aortic arch or its great branches, such as the subclavian or carotid, closely follow the time-pressure curve of left intraventricular pressure during the phase of ejection, and so are of importance in man as an index of the action of that chamber. This type of pulse is called the *central arterial pulse* to distinguish it from the *peripheral arterial pulse* as recorded in smaller arteries, such as the radial.

The differences between a *central* pulse as recorded in arteries such as the subclavian, and an intermediate or terminal pulse given by arteries such as the carotid, radial or dorsalis pedis * are due to the fact that the contour of the pulse, as it occurs at the aortic arch, is modified in its transmission to the periphery by friction, by incidental vibrations and by interference due to reflected waves. The central pulse, as recorded in Fig. 420 from the subclavian artery, shows the following features :

(1) An oscillation between A and B, due to the auricular systole (transmitted).

(2) A second elevation between B and D which corresponds with the isometric part of the ventricular contraction. (1) and (2) are probably transmitted vibrations.

* WIGGERS. *J. Amer. Med. Ass.,* 1915, 64, 1485.

(3) At D the semilunar valves open, and blood is rapidly ejected into the aorta, giving the main pulse between D and G. The sudden rise of pressure in the aorta sets the column of blood within the elastic vessel into vibration, giving the overshoot at E and the return wave or anacrotic halt, E F.

(4) The arterial curve now follows the intraventricular pressure (*vide* Fig. 348, p. 581), the flow of blood continuing quickly up to G, but then slowing off during the rest of the ventricular systole to H (Fig. 420).

(5) At the beginning of ventricular diastole there is a rapid backward movement of the blood towards the heart, causing the *incisura*, H I, and brought up rapidly by the closure of the aortic valves. The rebound of the blood from the valves causes one or two secondary elevations, K, after the incisura.

(6) The curve then falls gradually until the next ventricular systole supervenes, sometimes with slower waves at M.

The peripheral pulse gives similar features to the central pulse, but modified by the conditions mentioned above. The preliminary and anacrotic oscillations disappear (though the anacrotic oscillation may be counterfeited by a momentum swing of the lever in the ordinary imperfect sphygmograph); the incisura becomes less sharp, and is replaced by the *dicrotic* dip, and the wave K is replaced by the sometimes very conspicuous *dicrotic* wave; the main upstroke is delayed and its rise more gradual, while the amplitude of the whole curve is usually diminished. These changes are more marked as the periphery is approached, until finally in the smallest arteries and in the capillaries the pulse may disappear altogether.

Fig. 421 represents a pulse curve taken from the radial artery by means of a sphygmograph. It shows the anacrotic limb, or percussion wave, *a–b*, a peak at *b*, and a slower downstroke, or catacrotic limb which is broken by secondary waves, the most conspicuous of these being the *dicrotic wave*, with its preceding *dicrotic notch*, *c*. This sphygmogram should be compared with the curves, taken by more perfect methods, in Fig. 420, so as to appreciate the deformation by ordinary sphygmographs of the real curve of expansion of the arterial wall.

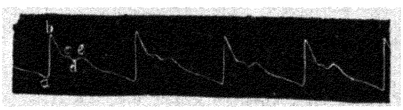

FIG. 421. Pulse Curve from Radial Artery.

The pulse curve gives approximate information as to the condition both of the heart and the arterial system. Thus, the period between the beginning of the primary elevation and the dicrotic notch corresponds to the outflow of blood from ventricle to aorta. A large pulse curve does not necessarily indicate a big output, since the expansion of the artery is determined not only by events occurring in the aorta but also by the expansibility of the artery and the resistance in the peripheral branches.

The general form of the pulse curve varies with changes in the heart, in the arteries and in the peripheral resistance. Thus, some sphygmograph curves may present secondary elevations on the ascending part, and are called *anacrotic*.

A curve of anacrotic type is normally seen in the subclavian artery (Fig. 420), but in the radial artery usually only in cases in which, owing to aortic stenosis, there is an ascending plateau in the intraventricular pressure tracing. When the peripheral resistance is high, or when an extra large amount of blood is thrown into the aorta at each stroke of the heart (*e.g.* by slowing of the rate), the aortic pressure rises very abruptly at the commencement of the ejection phase, and then more slowly so long as blood is flowing

in, and we get an ascending systolic plateau and an anacrotic pulse. If the pulse rate is quickened the anacrotic wave disappears.

The production of the dicrotic elevation is favoured by any influence which increases the distensibility of the arteries or causes the primary elevation of the pulse to be extensive and sharp. Thus it is much more pronounced in young people than in old people whose arteries have become rigid. When the peripheral resistance is low through relaxation of the arterioles, and the heart is beating forcibly, as in fever, and also to some extent after alcohol, the dicrotic elevation becomes so large that it may be felt at the wrist, and the mistake has been committed of taking the dicrotic wave for a normal beat, and so doubling the actual rate of the pulse.

REFLECTED WAVES. It has been suggested that, in the production of such a marked dicrotism, reflection from the periphery may play an important part. With a high blood pressure and rigid arteries, a reflected wave will travel back very quickly and will tend to add itself to the primary wave. With a low blood pressure, dilated arteries, and the output of the heart thrown rapidly into a relatively empty arterial system, the primary wave will rise and fall very rapidly, and the reflected wave will travel back along the arteries more slowly, so that its main effect might be to add to the dicrotic elevation normally proceeding outwards from the heart towards the periphery. The Fig. 417 VI would represent the condition as it is found in the femoral artery under normal circumstances when, according to Frank, the reflected wave adds to the height of the primary wave. In Fig. 417 V the reflected wave 'a' would tend to add to any dicrotic elevation present at this point, and might represent the relation existing in the arterial system with relaxed arteries and a heart beating forcibly, but throwing out only a small amount of blood at each beat.

It would seem that the central pulse can be referred entirely to events taking place in the heart during systole or at the beginning of the aorta at the commencement of diastole; and there is no reason to assume the co-operation of waves reflected from the periphery to explain any of the secondary waves observed. The effect of the dispersion of the sudden wave of pressure started in the aorta along an elaborate system of elastic tubes must be to diminish the rapidity of onset of each primary wave, and therefore to diminish the secondary vibrations of the curve. In an elastic system of tubes such as the arterial system, there are factors at work analogous in many respects to those responsible for the deformation of the curve given by an imperfect manometer.* These would be of two kinds—viz. oscillations of the column of fluid within the stretched arterial wall, and the reflection of waves from different points in the periphery. Many of these reflections will interfere with and annul one another. But in the arterial system there are certain points from which distinct reflections of waves can be expected—*e.g.* in the circle of Willis, at the bifurcation of the aorta into the two iliac arteries, and in the superficial and deep arterial arches in the hand and the foot. We have distinct evidence that such waves are set up and modify the form of the pulse in the femoral and brachial arteries and their branches. Thus in Fig. 420 V-VII, the primary rise of pressure in the femoral artery is higher than even the primary rise in the subclavian. This condition of things is explicable only on the assumption of a reflected wave passing back along the artery just after the passage of the primary wave, so that the two are summated. In the same way, although the dicrotic depression in the curve (Fig. 420, VII) is no doubt mainly the propagated effect of the incisura observed in the aortic pulse, it is probably deformed, and the subsequent elevation exaggerated, as a result of reflection of some wave from the periphery. The occurrence of reflected waves may serve to explain why the systolic pressure in the femoral artery is found higher, and the diastolic pressure lower, than in the brachial. The femoral artery being more rigid than the brachial, and the peripheral resistance more definitely localised, reflected waves occur in the artery at so short a time after the primary wave has passed down, that there is summation of the two waves, with production of a higher maximum and a lower minimum than was present in the waves as started in the aorta. If the leg be plunged into hot water, so as to dilate all its arterioles, this difference between the arm and the leg systolic pressures disappears. The varying development of reflected waves on the two sides may also explain why the systolic pressures in the two arms are rarely found to be identical.

* FRANK. *Z. f. Biol.*, 1905, **46**, 441.

Bramwell and A. V. Hill have pointed out * that, as the pressure rises rapidly in the artery at each pulse and the wall becomes more rigid, there is a corresponding increase in the velocity of propagation of the wave along the artery which would tend to make the rise steeper towards the periphery. This will be more marked the more rigid the arterial wall, and may account for the primary elevation in the big arteries of the legs being higher than in the more extensible arteries nearer the heart.

As the pulse wave travels along it changes in form, and under certain conditions part of the wave front becomes steeper, and eventually unstable. The result is a turbulent motion which may be compared with the formation of breakers in water waves on a shore. Such a change gives a peculiar character to the water-hammer pulse and is also responsible for the cracking character of the Korotkov sounds when the pressure on the armlet is just above the diastolic pressure.†

The *velocity of the pulse* wave can be measured by taking simultaneous tracings from two arteries separated by some distance from one another, such as the femoral artery and the *dorsalis pedis*, or from the carotid and radial arteries. In healthy individuals the velocity varies between 5 and 9 metres per second. The more rigid the arteries and the higher the arterial pressure the greater will be the velocity, so that the velocity of propagation gradually increases with advancing age,‡ and is higher in the arteries of the lower extremities than in the more distensible arteries of the arm.

The length of the pulse wave can be found by multiplying the velocity of transmission by the time occupied by the wave in passing any given point

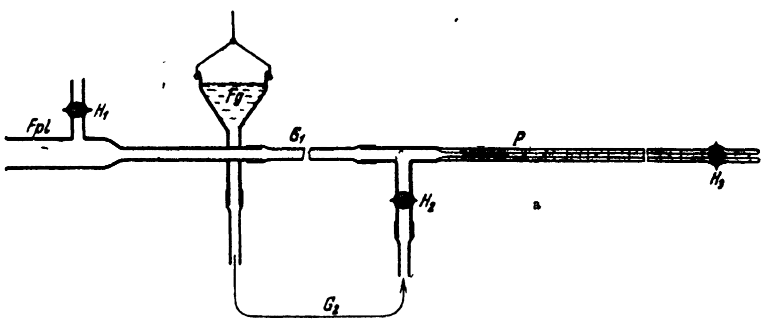

FIG. 422. Finger plethysmograph. The finger is placed airtight in *Fpl*, which is filled with warm water from *Fg*. The pulsations of the water meniscus in the capillary *P* are recorded photographically. (GOETZ. *Pflüger's Arch*, 1935, **235**, 271.)

(0·8 sec.), so that if the velocity of transmission be taken as 7 metres per second, the length of the wave is about 5·6 metres. The pulse wave thus begins at the periphery long before it has been completed in the aorta.

THE VOLUME PULSE IN ORGANS. Each heart beat, by causing a pulsatile expansion of the arteries throughout the body, results in the organs momentarily receiving blood faster than the veins carry it away. Hence there is a pulsating distension of all the vascular organs of the body, which may be graphically recorded by a delicate plethysmograph, one pattern of which, suitable for use with a finger, is shown in Fig. 422. The form of the volume pulse of the finger is seen to be similar to that of the radial pulse, though nearly 0·1 second behind it in time (Fig. 423).

* BRAMWELL and HILL. *J. Physiol.*, 1923, **57**, Pr. lxxiii.
† BRAMWELL. *Lancet*, 1937 (ii), 366.
‡ BRAMWELL, HILL and MCSWINEY, *Heart*, 1920, **10**, 3; BRAMWELL and HILL, *Proc. Roy. Soc.*, 1922, **93**; HICKSON and MCSWINEY, *J. Physiol.*, 1924, **59**, 217.

When the tissues are tense with inflammatory œdema, the pulsatile distension causes the familiar throbbing pain because the tension is raised at each pulse wave, and pain fibres thereby stimulated.

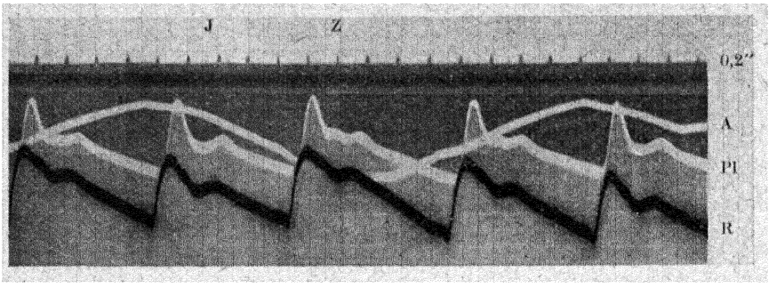

FIG. 423. Volume pulse of finger (Pl) recorded together with the radial pulse (R) and respiration (A) (inspiration upwards). Note the different character of the dicrotic waves at inspiration and expiration. (GOETZ. *Pflüger's Arch.*, 1935, **235**, 271.)

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THE CIRCULATION THROUGH THE CAPILLARIES

The circulation through the minute surface vessels of the skin in man, and in other tissues can readily be observed when a microscopic examination is made by reflected light. The skin is for this purpose covered with cedar oil or liquid paraffin (Fig. 424).

The capillary circulation is best demonstrated by examining under the microscope the tongue of the frog or the web of the frog's foot, when a network of vessels is seen, consisting of small arteries, capillaries and veins. The flow of blood in the arteries is rapid, whereas in the veins it is slower, and is slowest in the capillaries. In the capillaries the flow is from arteries to veins, though, on account of the reticular arrangement of these vessels, the direction of the stream through them is not quite constant. If a group of capillaries be watched for some time, the blood may at first hurry through a number of them with rapidity ; the flow then becomes slower and may quicken up to a moderate pace again. These variations in the capillary flow are probably associated with spontaneous alterations in the condition of contraction of the small arteries supplying the group of capillaries. It is easy to observe that the arterial flow is pulsatile, the pulsation being reduced, or disappearing in the capillaries and veins. Another difference between the circulation in these three kinds of vessels is to be found in the condition of the peripheral zone. In the arterioles the blood stream is divided into two parts, the peripheral stream, consisting only of colourless plasma with occasionally a stray leucocyte—and an axial stream, in which all the red blood corpuscles are being hurried along. In the veins there is a similar peripheral plasmatic zone, but here we find regularly scattered leucocytes which travel rather more slowly than the axial stream of red corpuscles. The formation of this axial zone is purely mechanical, and may be imitated in any fluid containing in suspension particles whose specific gravity is some-

what higher than that of the fluid. In the capillaries there is no separation of the two zones, since the lumen of these vessels is so narrow that often the corpuscles, passing in single file, are squeezed out of shape as they progress. The corpuscles are evidently elastic structures, and may be seen to bend if they impinge on the dividing point of two capillaries, before they are finally swept off by the stream into one or the other.

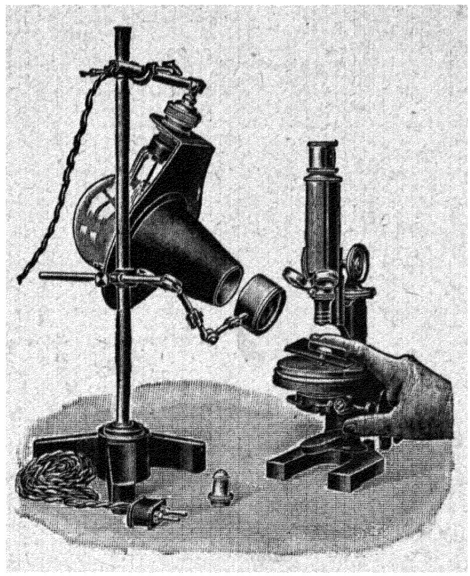

FIG. 424. Arrangement of Illumination for Viewing Skin Capillaries.

The capillary wall is composed of a single layer of elongated flattened cells which present little resistance to the passage through them by diffusion of dissolved substances. In this way the tissue cells obtain oxygen from the red blood corpuscles and nutriment from the plasma, and give off to the circulating blood carbon dioxide and other products of their metabolism. There is evidence that a sort of "contractility," due to a swelling of the nuclei of their endothelial cells when stimulated, is a universal attribute of the capillaries, and must play an important part in determining the amount of blood flow through an organ in accordance with its needs. In resting tissues many of the capillaries are so constricted as to allow of no flow at all.

The average length of a capillary is between 0·4 and 0·7 mm. The velocity of blood flow can be directly determined by observing under the microscope the time taken by any given corpuscle to travel a measured distance on the microscope stage. The mean velocity determined in this way varies from about 0·5 to 0·8 mm. per second.

The blood pressure in the capillaries may be measured approximately by applying pressure to the outer surface of the skin or mucous membrane by means of a Recklinghausen capsule (Fig. 403) and noticing the point at which blanching of the surface commences. The errors of this method are considerable. The pressure in the capillaries, as found by this method, is low, and necessarily varies with the position of the part under investigation, *i.e.* with the hydrostatic pressure. It is raised, often greatly, when there is a dilatation of the arterioles, and lowered when these constrict.

Capillary pressures have also been measured in man by Carrier and Rehberg as follows : A fine glass capillary is filled at the point with saline solution and pushed into a skin "capillary" which is under observation under the microscope. Attached to the other end of the capillary tube is a water manometer, by means of which a definite pressure is applied to the column of saline solution in the capillary glass tube. When the blood capillary is pierced, blood will flow into the glass capillary if the capillary pressure is higher than that in the water manometer ; if lower, no blood will enter ; if the same, blood will pulsate in and out of the glass capillary at each heart-beat.

In man it has been found that the capillary pressure in the skin of the finger varies with the position of the hand with reference to the heart, and

may be 40 cm. H_2O, or more, or as little as 4 or 5 cm.; but, however high up the hand is placed, it never falls below a certain level, of about 4 to 5 cm. The reason for this is that the veins collapse and restrict the flow when the hand is held in such a position that the venous pressure becomes negative. The capillary pressure is thus always maintained at a positive value, which varies according to the position. In the feet the capillary pressure is normally prevented from attaining very high values, owing to the presence of valves in even the smallest veins, and to the occurrence of constant small muscular movements, which, by forcing the blood along these veins, enables them to act as a kind of pump. The pressure gradient along a capillary is considerable. Landis * found the average pressure at the arterial end of skin capillary loops to be 32 mm. Hg and that at the venous end 12 mm. Hg.

Owing to the fact that a considerable resistance—that of the arterioles—lies between the capillaries and the arteries, the pressure in the capillaries must stand in much closer relationship to that in the veins than to that in the arteries. One cannot therefore argue that a fall of arterial pressure necessarily involves a fall of capillary pressure in all parts of the body. We can only judge of changes in the capillary pressure by simultaneously taking the pressures in both the afferent and efferent vessels. If these both rise or fall together we may be certain that the capillary pressure also rises or falls. When the arterial and venous pressures move in opposite directions, it is difficult to say what alterations, if any, will be produced in the capillary pressure.

The resistance to the flow of blood through the capillaries is determined by the internal friction, *i.e.* the viscosity of the blood; as already explained (p. 648), when circulating in the blood capillaries, this is only about twice that of water. The velocity of flow through a capillary bed varies directly as the driving (arterial) pressure, provided this is above 20 mm. Hg, below which little flow occurs. The chief seat of the resistance in the vascular system is in the arterioles, and it is in this region that the chief fall of pressure occurs.

No part of the circulation shows greater variations than the capillary system. We must think of this as a vast irrigation system—the greater part of which is closed under normal circumstances, and open only when the tissue requires a large increase in the supply of blood. In muscle the capacity of this irrigation system may be increased 750 times during activity. Such changes (if of widespread occurrence) will affect arterial pressure by their influence on the total capacity of the vascular system as well as by alterations in the peripheral resistance.

Capillary Fragility. The walls of the capillaries are lined continuously with endothelial cells, and do not normally allow even the proteins of the plasma to pass through. When subjected to a sufficient pressure, however, the walls give way, and then blood escapes into the surrounding tissues, causing a local discoloration. When seen in the skin such patches appear as red spots called *petechiæ*. In certain conditions (*v.* p. 861) such as scurvy, these petechial hæmorrhages occur very much more easily than in normal states. The tendency of the skin capillaries to rupture can be found by means of Göthlin's fragility test. A sphygmomanometer armlet is placed round the upper arm, and raised to, and kept at, 50 mm. Hg for fifteen minutes. The armlet is then removed and the skin of the antecubital fossa examined for petechiæ. Not more than eight should be present in a circle of 6 cm. diameter, when examined in a strong light.†

* LANDIS. *Heart*, 1930, 15, 209.

† BELL, LAZARUS and MUNRO. *Lancet*, 1940, 239, 154.

THE FLOW AND PRESSURE OF BLOOD IN THE VEINS

In the veins there must be a constant decrement of pressure as we pass from the periphery towards the heart. Owing to the fact that no appreciable resistance lies between the veins and the heart, the pressure gradient necessary to maintain a constant flow through these vessels is very small. Thus, in the horizontal posture, the pressure in the femoral veins may be from 5 to 10 mm. Hg., and in the external jugular vein from 0 to — 2 mm. It is convenient for clinical purposes to observe the extent to which the external jugular vein is filled with blood when the patient lies down with the head raised. The vein then acts as a manometer, and is seen to be distended to a level which is normally 0 to 2 cm. below a horizontal line passing through

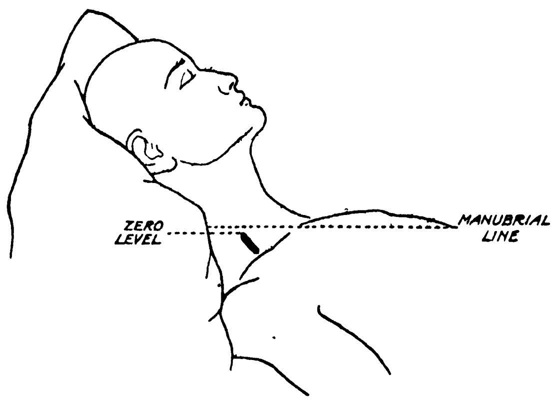

FIG. 425. Level of filling of external jugular vein. (LEWIS.)

the junction of the sternum with the manubrium (Fig. 425). The pressure in the great veins near the heart is generally below that of the atmosphere owing to the aspiration of the thorax, and becomes still lower during inspiration. Opening the thorax, therefore, causes a rise of pressure in all the large veins. In the latter, the pressure is lowered by vigorous action of the heart pump and raised when this fails in any way. In the peripheral veins the pressure is more dependent on the flow through the corresponding arteries. If an artery of a limb be ligatured, the pressure in the small veins of the limb sinks until it is reduced to the pressure in the nearest large trunk in which a flow of blood continues.

If a vein is ligatured, the blood usually finds its way by anastomotic channels into another vein. But if the occluded vein is large, one result will be a rise of capillary and venous pressures distal to the occlusion, and this will continue until adequate anastomoses have been opened up. When all the veins leading from a part, such as a limb are compressed, the capillary and venous pressures behind the occlusion will rise until they either overcome the resistance, or finally equal the arterial pressure. Thus, if a sphygmomanometer armlet be placed on the upper arm, and inflated to 50 mm. Hg, the venous pressure in the arm veins will rise until it exceeds 50 mm. Hg, so that all the veins are engorged, and if the pressure be raised to 70 mm. the venous pressure will rise accordingly, and so on.

The Venous Pulse. Each cardiac cycle causes variations in the pressure in the great veins next the heart in two ways :

(1) By the transmission backwards along the veins of the alterations in the intra-auricular pressure.

(2) By the diminution in the volume of the heart in consequence of the expulsion of its blood along the arteries with each heart beat.

On this account the jugular veins show pulsations with each heart beat which are complex in character and resemble those occurring in the auricle (*vide* p. 582). They can be recorded by the use of the polygraph (Fig. 419, p. 661), by applying an open capsule over the lower part of the external jugular vein, and leading to the recording tambour. Jugular tracings (Fig. 426) give information as to the time at which auricular systole occurs, as compared with ventricular systole, which can be deduced from the arterial tracing. It will be seen that each heart beat gives rise to three positive waves of pressure within the veins. These three undulations are analogous to those given in Fig. 350, p. 583, as occurring in the auricular tracing. We regard *a* as due to auricular contraction, *c* as the elevation due to closure of the

FIG. 426. Venous Pulse Tracing from Jugular Vein compared with the Arterial Pulse Tracing from the Radial Artery.

auriculo-ventricular valves, *v* as the elevation due to the accumulation of blood in the auricles during the ventricular systole. The curve *c* is probably due in part also to an impact transmitted from the great arterial trunks to the intrathoracic veins. These venous pulsations are much more marked in cases of heart disease, where there is overfilling of the venous system, often combined with incompetence of the auriculo-ventricular valves.

In cases of heart block (p. 575) the auricle and ventricle contract independently (complete heart block) or at a ratio of two or more auricular contractions to one ventricular. These altered conditions are shown on the jugular and arterial pulse tracings in which jugular tracings show the auricular contractions and the arterial tracings the ventricular contractions (Fig. 343, p. 575).

Valves. Besides the favourable influences exercised on the circulation through the veins by the aspiration of the thorax, a considerable part is played in the venous circulation by the emptying of the veins by pressure upon their walls. The adjuvant effect of movement on the circulation through the veins is rendered possible by the existence in these vessels of valves, which are semilunar folds of the intima projecting into their lumen, and so arranged that they allow the passage of blood only towards the heart. Two such folds are, as a rule, situated opposite to each other. Every movement of a limb, active or passive, causes an external pressure on the veins and empties them towards the heart. Thus in walking, each time the thigh is moved backwards the femoral vein becomes empty and collapses, and fills again as soon as the leg is brought forward to its former position.

When muscular movements become general, as in walking or running, the active compression of the veins thus brought about plays an important part in hurrying the blood into the right heart, so that the output of this organ is increased.

Since the blood in the vessels is subject to the influence of gravity, we might expect to find that the pressure in the veins of the foot was equal to the pressure in the veins at the level of the heart *plus* the pressure of the column of blood between these veins and the heart, *i.e.* about a metre of blood, but this is not the case. The pressure, indeed, in the veins of the foot is but little higher than that in the veins of the hand, as L. Hill has shown. When a man assumes the upright position, the arteries of the leg and foot contract until, under the combined influence of the heart's contraction and gravity, the blood supply to the capillaries is just adequate. The return of the blood from the dependent parts cannot be ascribed entirely to the heart beat, but is largely due to the contractions of the muscles of the limb, which press on all the deep and superficial veins, and in virtue of the valves force the blood contained therein past Poupart's ligament into the abdomen. This explains why it is so difficult to stand for any length of time without moving, and emphasises the need of moderate exercise for the maintenance of a normal circulation.

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CHAPTER XXXIV

LOCAL REGULATION OF BLOOD FLOW

THE NERVOUS CONTROL OF THE BLOOD VESSELS

Every organ of the body requires an increased blood supply during activity, which means that blood must be diverted from the inactive to the active tissues. This is chiefly effected through the central nervous system, though local and chemical mechanisms also play some part in the process.

THE VASCULAR NERVES. Our knowledge of the nervous control of the blood vessels dates from the discovery, by Claude Bernard, that nerve fibres maintain them in a state of tonic constriction.* Bernard showed that if, in the rabbit, the cervical sympathetic on one side be divided, the vessels in the corresponding ear dilate. Vessels previously invisible come into prominence, and on account of the greater flow of blood produced, the ear on the side of the section becomes warmer than the normal ear. If the head end of the divided sympathetic nerve be stimulated, all the vessels of the ear contract, and the ear becomes colder than the other. The fact that the dilatation of the vessels produced by section lasts for a considerable time after any irritant effect of the section must have passed off, shows that the ear vessels are under the influence of "tonic," *i.e. continual*, constrictor impulses arising in the central nervous system and proceeding to them along the cervical sympathetic.

The paralysis of the ear vessels, though lessening the local resistance to the flow of blood, affects too small a vascular area to have any marked influence on the general arterial pressure. But if the spinal cord be divided on a level with the origin of the first dorsal nerve, or higher, a wide area is deprived of vasoconstrictor control and the arterial pressure sinks considerably. The heart after the section beats more rapidly than before, so that the fall of pressure must be ascribed to a change affecting the blood vessels and lowering the resistance to the flow of blood. Since a maximal effect on the blood pressure is produced by section of the cord at this level, one may conclude that the tonic constrictor impulses to a large proportion of the vessels of the body pass down this segment of the cord on their way to the arterial walls.

The source of these impulses may be made out by studying the effect of sections through different levels of the nervous system. Division of the cord at, or below, the second lumbar nerve causes no effect on the blood pressure. On making a section above the first lumbar nerve, the effect produced increases progressively until the first dorsal roots are reached, where it is maximal; stimulation of the lower end of the cut dorsal cord causes widespread vascular constriction and a large rise of blood pressure.

We must therefore conclude that all the vessels in the body are kept in a state of tonic contraction by impulses arising from a higher centre, travelling down the cord as far as the dorsal region, and then passing out of the cord by the dorsal and upper lumbar nerves.† This conclusion is con-

* Cl. Bernard. *C. R. Soc. Biol.*, 1852, **4**, 168.

† Gaskell. *J. Physiol.*, 1885, **7**, 1.

firmed by the fact that, whereas stimulation of the ventral roots of the cervical, lower lumbar and sacral nerves has no influence on the blood pressure, a rise of arterial pressure can be obtained by stimulating any of the ventral roots from the first or second dorsal to the second or third lumbar. The same effect is produced by stimulation of the white rami communicantes from these roots to the sympathetic system, by excitation of the sympathetic system itself, or of the splanchnic nerve.

Since the plain muscle of the blood vessels exhibits an automatic tonus, complete nervous control of these tubes can be secured only by the provision of two sets of nerves: one set—constrictor or motor—which will increase the state of constriction of the vessels; another set—inhibitor or dilator—which will diminish the tone of the arteriole muscle, and cause vascular dilatation. Our knowledge of the existence of this second class of nerve fibres to the vessels we owe also to Claude Bernard,* who observed that stimulation of the chorda tympani nerve increased the blood flow through the submaxillary glands.

Criteria of Vasomotion. In order to investigate the state of tone of the small blood vessels it is necessary to have means for investigating the condition of the blood flow through different parts and organs of the body. It is obvious that if the arterioles A in the organ B dilate (Fig. 427), the first effect is a diminution of the resistance to the flow of blood into the capillaries beyond. If the arterial pressure in the trunk C remains constant, a local diminution of resistance in A will at once determine an increased flow of blood through the arterioles, and a rise of capillary pressure. If the organ is distensible, the increased pressure in the arterioles and capillaries will cause a swelling of the whole organ. But the same effect on intracapillary pressure, and therefore on the volume of the part, may also be caused by obstruction to the flow of blood from the veins V. It is only when, the arterial pressure in C remaining constant, dilatation of an organ is *accompanied by an increased blood flow through it* that it may be taken as an expression of vasodilatation in the arteries with which it is supplied.

We can therefore use the following criteria for the occurrence of a vasodilatation (and their converses for vasoconstriction), in the arterial supply to any part or organ:

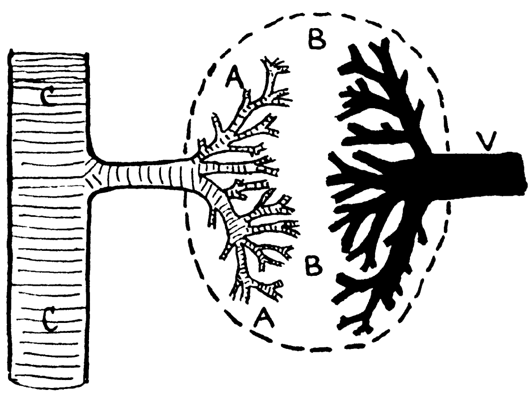


FIG. 427

(1) If the surface of the part is translucent, the increased filling of the capillaries will cause redness.

(2) The increased size of the vessels will cause an *increase in the volume* of the organ concerned.

(3) An increased velocity of blood flow will usually raise its temperature.

(4) An increased velocity in the arteries going to the part may be interpreted as due to vasodilatation.

(5) The increased flow may also be detected by measuring the total volume of blood which flows from the vein in a given time.

* BERNARD. *C.R.*, 1858, **46**, 159.

Of these methods the two most used are those based on determination both of the volume of the part, and of either the arterial inflow to, or of the venous outflow from, the part. A fallacy may arise, however, unless means be taken to ensure that the general arterial pressure remains constant during the experiment. A rise of arterial pressure will cause a *passive* expansion of the vessels and of the part supplied, and also increased velocity of blood flow through the part. In all cases, therefore, it is necessary to record the arterial pressure. We may take as an instance an experiment on the blood supply to the kidney.

For this purpose we may determine the change in the volume of the organ by the use of a *plethysmograph*.

Sharpey Schafer's plethysmograph * (Fig. 428), which can be adapted to almost any organ of the body, is made of Stent's wax previously moulded to the size of the organ whose volume is the object of investigation. In one side of the box a depression is left,

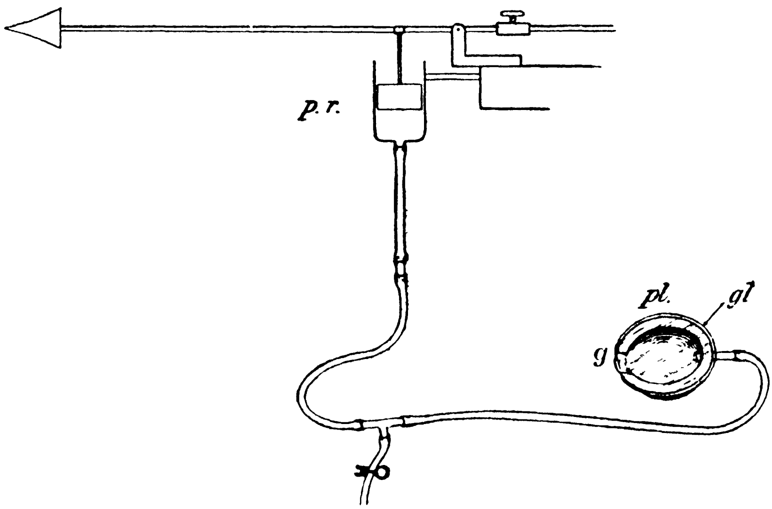


FIG. 428. Diagram of Sharpey Schafer's Air Plethysmograph *pl*, the plethysmograph, covered by a glass plate, *gl*; the vessels pass in through the groove *g*, *p.r.*, the piston recorder. (From SHARPEY SCHAFER'S "Experimental Physiology," Longmans, Green)

sufficient to accommodate easily the vessels, nerves (and ureter) going to the organ The plethysmograph is covered with a glass lid which is made air-tight by means of vaseline, the space between the lid and the vessels being also packed with cotton-wool and vaseline. A tube from the plethysmograph leads to a piston recorder. Every variation in the volume of the organ causes a movement of air into or out of the plethysmograph and thus gives rise to a corresponding movement of the recording lever.

The kidney being placed in some such apparatus, and the arterial pressure being recorded, as in Fig. 429, the peripheral end of the anterior root of the tenth dorsal nerve was stimulated; this was followed by a rise of blood pressure together with a diminution of the kidney volume. The increased blood pressure would by itself tend to force more blood into the kidney and so increase its volume. Since the kidney volume diminished there must have been active contraction of the arterioles of the kidney. This contraction of the vessels must have taken some part at any rate in the rise in general blood pressure. If the plethysmograph had been used alone, it

* SCHAFER and MOORE. *J. Physiol.*, 1896, 20, 1.

would have been impossible to determine whether the shrinkage of the kidney might not be due to a lowering of arterial pressure, in consequence of vasodilatation occurring elsewhere or in consequence of the failure of the heart's activity. On the other hand, without the volume record it would only have been possible to infer that there was increased peripheral resistance somewhere or other in the body.

Instead of taking the volume of the kidney, we might have determined the blood flow through its vessels; this would have been found to be diminished by the stimulation. The method is of great value in dealing with the circulation through small organs, such as the submaxillary glands. In such a case it is usual to hinder or prevent the clotting of the blood by the preliminary injection of heparin. and then, after placing

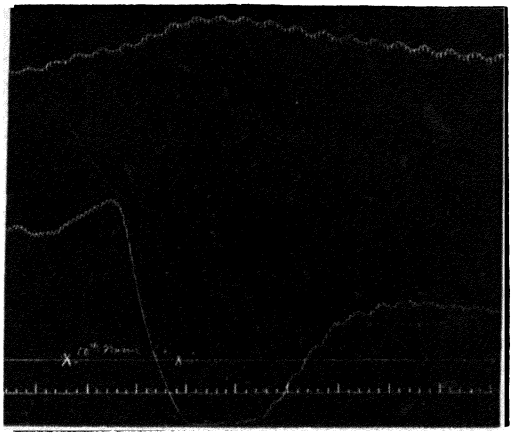


FIG. 429. Simultaneous Tracings of Carotid Blood Pressure and Volume of Kidney. Between × and × the peripheral end of the divided tenth dorsal nerve was stimulated. Time-marking = seconds. (BRADFORD.)

a cannula in the efferent vein of the organ, to allow blood from the cannula to flow into an automatic measuring apparatus.

For recording the arterial inflow the Rein thermostromuhr is now often employed (p. 655).

Course of the Vasoconstrictor Fibres. The vasoconstrictor fibres belong to the sympathetic system.

The position of the synapses on the path of the fibres is shown by the use of nicotine (Langley).* After injection of nicotine, stimulation of the pre-ganglionic fibres is without effect, although the normal results follow stimulation of the post-ganglionic non-medullated fibres.

By this method it has been determined that all the vasoconstrictor nerves of the body leave the spinal cord by the ventral roots of the spinal nerves from the first dorsal to the third or fourth lumbar inclusive. From the roots they pass by the white rami communicantes to the ganglia of the sympathetic chain. Here they take different courses according to their destination.

The vasoconstrictor nerves to the periphery of the body (Fig. 430), including the skin and muscles, pass by the grey rami communicantes to the mixed spinal nerves and are distributed with the ordinary motor and sensory nerves.† The distribution is strictly unilateral, stopping abruptly at the

* LANGLEY. *J. Physiol.*, 1890, **11**, 123.
† GILDING. *J. Physiol.*, 1932, **74**, 34.

mid-line, in spite of the fact that the blood vessels themselves anastomose freely across the mid-line. It would seem therefore that, so far as the skin supply is concerned, the periarterial plexus described by Woollard * is not concerned with the transmission to a distance of vasoconstrictor impulses.

The fibres to the head and neck leave by the first four thoracic nerves, and pass to the superior cervical ganglion. Here they end, and the impulses are carried by a fresh relay as non-medullated fibres on the walls of the carotid artery and its branches.

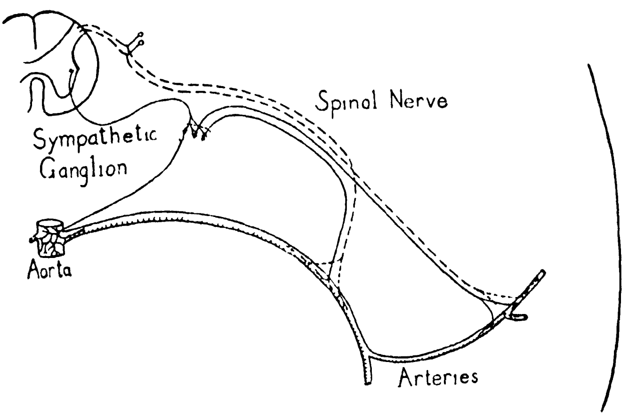


FIG. 430. Diagram showing the distribution of nerves to the arterial vessels. Sympathetic fibres continuous lines. Spinal afferent fibres dotted lines. (RANSON. *Ann. Int. Med.*, 1933, **6**, 1013.)

The constrictors to the fore limb in the dog leave the cord by the fourth to the tenth thoracic nerves. The fibres run to the stellate ganglion, where they all end in *synapses*. The impulses are carried on by the grey rami to the nerves which make up the brachial plexus, and run down in the branches of this to the vessels of the fore limb.

The constrictor impulses to the hind limb in the dog arise from the nerve roots between the eleventh dorsal and third lumbar roots, end in connection with cells in the sixth and seventh lumbar and first and second sacral ganglia of the sympathetic chain, whence the impulses are carried by grey rami to the nerves making up the sacral plexus.

The chief vasoconstrictor supply to the abdominal viscera is the *splanchnic nerve*. This receives fibres of the white rami from the lower seven dorsal and upper two or three lumbar roots, the latter fibres often taking a separate course as the lesser splanchnics. The fibres pass through the sympathetic chain of the thorax without interruption, and for the most part have their cell station in the collateral ganglia, especially the cœliac ganglion, whence a thick mesh-work of non-medullated fibres is distributed along all the vessels of the abdominal viscera. The area of the vessels innervated by this nerve is so large that section of it on both sides causes a definite fall in the arterial pressure.

CONSTRICTORS TO VEINS. Often the veins are also supplied with sympathetic vasoconstrictor fibres † ; their functions are less conspicuous than those of the arteries, but must be borne in mind, particularly in the interpretation of plethysmographic tracings.

Vasodilator Nerves. Since the arteries are usually in a condition of moderate contraction, a dilatation can be brought about by an inhibition of the normal constrictor impulses proceeding to the vessels from the vasomotor centre. We find, however, in many parts of the body, also a vasodilator nerve supply.

* WOOLLARD. *Heart*, 1926, **13**, 319.
† HOOKER. *Amer. J. Physiol.*, 1918, **46**, 591 ; DONEGAN. *J. Physiol.*, 1921, **55**, 226.

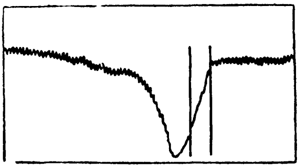
There are three types of vasodilator fibres, viz. parasympathetic, sympathetic and posterior root or 'antidromic' fibres. The clearest examples are found in parasympathetic nerves.

PARASYMPATHETIC VASODILATORS. If the *chorda tympani* nerve be cut, no change is evident in the blood vessels of the submaxillary gland. But if its peripheral end be stimulated, there is secretion of saliva, and all the blood vessels are largely dilated.* In consequence, the blood rushes through the capillaries so abundantly that the blood flowing from the vein is arterial in colour. Other examples of vasodilator nerves are the *small petrosal* nerve to the parotid gland, the *lingual* nerve to the blood vessels of the tongue, and the *pelvic visceral* nerves to those of the genitalia.

SYMPATHETIC VASODILATORS. Some of the fibres of the sympathetic system are vasodilator in function. This is notably the case for the sympathetic supply to the coronary vessels of the heart. Also, in the dog, dilatation of the vessels of the soft palate and gums can be produced by stimulation of the cervical sympathetic of the same side, or of the stellate ganglion, or its *rami communicantes*. Again, in the muscles of the dog, sympathetic dilators preponderate. In other species the muscles have only sympathetic vasoconstrictors, as in rabbit and monkey. Usually, special conditions must be present in order to render possible the demonstration of sympathetic vasodilators. Thus, stimulation of the splanchnic nerves may cause a fall of blood pressure, provided that the constrictor fibres, which predominate, have been paralysed by the previous administration of large doses of ergotoxine.† Again, the presence of vasodilator fibres in the lumbar sympathetic chain supplying the hind limb in the dog can be demonstrated when this is stimulated, provided that some adrenaline is present in the circulating blood.‡ This effect is intensified by eserine and annulled by atropine. Hence it may be concluded that these vasodilators, though sympathetic, are cholinergic: they chiefly supply the muscles. In the cat the sympathetic vasodilators are few in number and are adrenergic.§

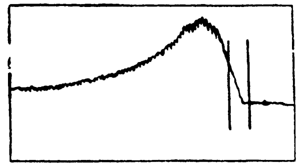
ANTIDROMIC VASODILATORS. In addition, there are in the mixed spinal nerves fibres which are vasodilator. Since, however, the mixed nerves to the limbs also contain constrictor fibres, chiefly to the skin, the effect of the constriction overpowers any effects due to dilator fibres. Moreover, the dilators apparently do not conduct any tonic influences to the blood vessels, so that the only effect of section of a mixed nerve is that due to the removal of the tonic constrictor influences, and the vessels in the area of distribution of the nerves therefore dilate.

A



Nerve Freshly Divided.
Constriction.

B



Nerve Four Days Degenerated.
Dilatation.

FIG. 431. Plethysmographic Tracing of Hind Limbs, showing effect of Stimulating the Sciatic Nerve on the Volume of the Limb, A, immediately after section of the nerve; B, four days after section. The nerve was stimulated between the two vertical lines. Curves to be read *from right to left*. (BOWDITCH and WARREN.

* CL. BERNARD. *C. R.*, 1858, **46**, 159.
† DALE. *J. Physiol.*, 1913, **46**, 294.
‡ BURN. *J. Physiol.*, 1932, **75**, 144.
§ BÜLBRING and BURN. *J. Physiol.*, 1935, **83**, 483.

Various methods have been employed to show the presence of dilator fibres in such a mixed nerve trunk. One method is to skin the limb, which is supplied almost entirely by constrictor fibres, whereby vasodilator responses are more easily revealed. Of other methods, the two chief are those depending on the unequal time taken for the two sets of fibres to degenerate, and on the varying excitability of the two sets of fibres to different kinds of stimulation.* Thus, if the sciatic nerve be cut, the primary dilatation of the vessels of the leg and foot passes off after two or three days. If, now, the peripheral end of the divided nerve be stimulated, dilatation of the vessels is brought about (Fig. 431). Apparently, the constrictor fibres degenerate before the dilator fibres, so that, at a certain period after the nerve section, only the latter respond to stimulation. On the other hand, it is often possible, even in the freshly cut nerve, to obtain dilatation by stimulating its peripheral end with induction shocks repeated at slow intervals—one to four per second, as shown in Fig. 432.

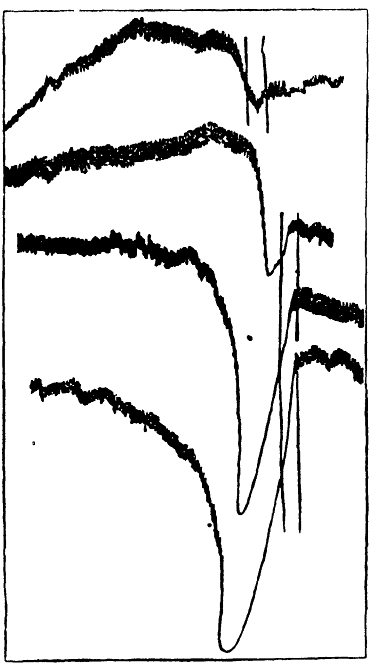


FIG. 432. Effect on the Volume of the Hind Limbs of the Cat of Stimulating the Sciatic Nerve with Induction Shocks at Different Rates.

It will be noticed that with one shock per second there is hardly any constriction, but considerable dilatation, whereas with 64 shocks per second the only effect produced is vasoconstriction. Curves to be read *from right to left*. (BOWDITCH and WARREN.)

These limb dilator fibres are not connected with the sympathetic system, but with the *dorsal* nerve roots. It was shown by Stricker in 1876 that dilatation of the vessels of the hind limb could be produced by stimulating the dorsal roots of the nerves going to the limb, *i.e.* far below the point of origin from the cord of the constrictor fibres to the same part of the body. This was confirmed by Bayliss.† Stimulation of the dorsal roots, either before or after they have passed through their ganglia, causes dilatation of the vessels in the area of skin and muscle ‡ supplied by the roots, whether the stimulus be electrical, chemical, or mechanical (Fig. 433). This effect is not destroyed by previous section of the dorsal roots on the proximal side of the ganglia, showing that the fibres by means of which the dilatation is produced have the same course as the ordinary sensory nerves to the limbs. Since the impulses on artificial stimulation pass in a direction opposite to that taken by the normal impulses, Bayliss designated them *antidromic* impulses. The phenomenon of a nerve fibre functioning in both directions is without analogy in our knowledge of the other nerve functions of the body,

* BOWDITCH and WARREN. *J. Physiol.*, 1886, **7**, 416.
† BAYLISS, W. M. *J. Physiol.*, 1901, **26**, 173; 1902, **28**, 276.
‡ WYBAUW. *Arch. int. Physiol.*, 1938, **46**, 293.

and there is doubt as to the relation of the phenomena obtained by stimulation of the dorsal roots to those of vasodilatation in the normal state, as, *e.g.* to local stimulation by mechanical means or by irritants, such as mustard,

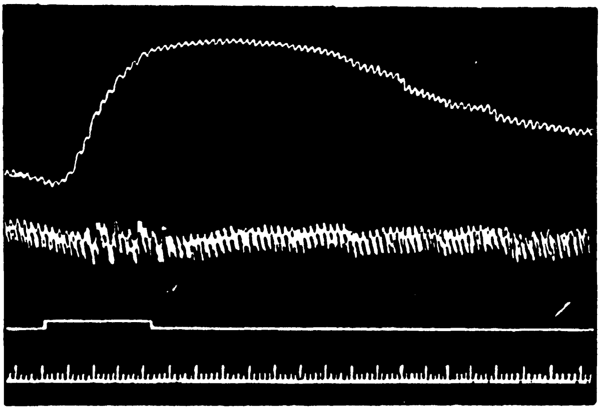


FIG. 433. Effect of Excitation of Peripheral end of the Seventh Lumbar Dorsal Root in the Dog. (BAYLISS.)

Uppermost curve, volume of left hind limb; next below arterial blood pressure; the third line marks the period of stimulation, bottom line, time-marking in seconds.

applied to the skin. There are undoubtedly centrifugal fibres in the dorsal roots (p. 241), but where these terminate is uncertain. The commonly accepted view of the normal vasodilator response which is effected through the posterior root fibres is that it is an axon reflex. If the dorsal nerve roots

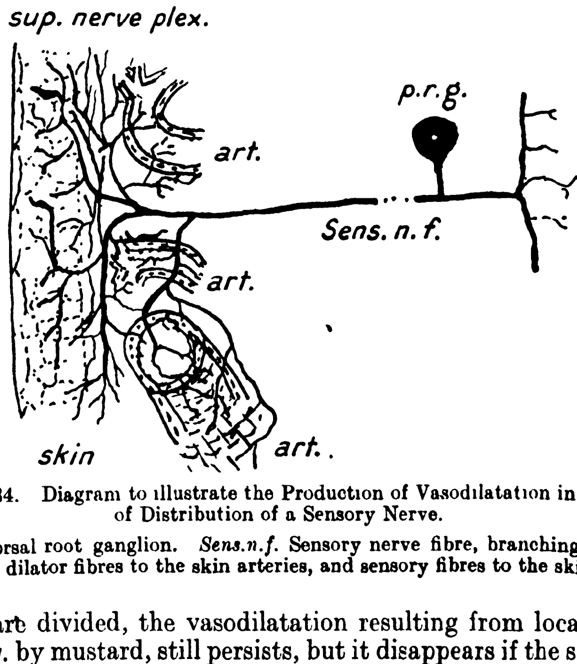


FIG. 434. Diagram to illustrate the Production of Vasodilatation in the Area of Distribution of a Sensory Nerve.

p.r.g. Dorsal root ganglion. *Sens.n.f.* Sensory nerve fibre, branching to supply dilator fibres to the skin arteries, and sensory fibres to the skin.

concerned are divided, the vasodilatation resulting from local irritation of the skin, *e.g.* by mustard, still persists, but it disappears if the section is made distal to the dorsal root ganglion and time allowed for the fibres to degenerate.

It also disappears if the skin is locally anæsthetised by injection of novocain. Hence it is assumed that the axons of some, presumably afferent, nerves branch, as shown in Fig. 434, one branch going to afferent receptors and another to the muscle cells of cutaneous arterioles. Afferent fibres of the trigeminal are believed to behave similarly.

However they reach the minute vessels, there is some evidence that the results there produced by the nerve impulses may be due to the liberation of potent chemical substances in the tissues of the locality. These may be histamine * or acetyl choline,† the former dilating capillaries, the latter the arterioles.

It may be noted that in certain inflammatory conditions in the dorsal root ganglia, there is reddening of the skin in the areas supplied by the respective roots, often followed by a vesicular eruption (Herpes Zoster) also confined to those areas.

THE VASOMOTOR CENTRE. The portion of the medulla concerned with the sending out of the tonic vaso-constrictor impulses is spoken of as the vasomotor centre. Section of the crura cerebri, or of the brain stem at the upper border of the fourth ventricle, leaves the blood pressure unaffected. Destruction of a small region of the medulla situated on each side of the middle line in the neighbourhood of the facial nucleus, *i.e.* in the forward prolongation of the lateral columns, after they have given off their fibres to the decussating pyramids, causes an immediate and maximal lowering of the blood pressure. Stimulation of a point, the fovea inferior on the floor of the fourth ventricle, and also often of the facial colliculus, causes a rise of blood pressure, while excitation of a point, the obex, near the calamus scriptorius, causes a fall. The former effect is probably due to excitation of the vaso-constrictor centre, and the latter has similarly been supposed to be the result of stimulation of a vasodilator centre.‡ The existence of a true vasodilator centre is doubtful, however, since there is evidence that stimuli applied to the depressor point act on some afferent tract which mediates reflex vasodilatation.§

The vasomotor centre is played upon by afferent impulses from the higher centres of the brain, but especially by afferent impulses travelling from all parts of the body, including those carried by the vagi from the viscera and by the carotid sinus nerve. Whether, in the absence of all afferent stimuli, the centre would be active is doubtful; all we know is that the sum of the stimuli arriving at the centre produces a state of average continued activity, which is responsible for the maintenance of arterial tone.

CHEMICAL EFFECTS ON CENTRE. An important factor for the maintenance of the tonic activity of the centre is the presence of carbon dioxide in the blood. If this be lowered by forcible ventilation of the lungs, so as to remove much carbon dioxide out of the blood, a profound fall of blood pressure occurs owing to paralysis of the vasomotor centre. On the other hand, anything which interferes with the gaseous exchanges of the centre, whether obstruction to respiration, the action of cyanide, or a failure of the blood supply, as by ligature of the cerebral arteries, calls forth an increased state of activity of the centre. This can be illustrated by observing the changes in the blood pressure produced in a curarised animal by the cessation of artificial respiration.

* LEWIS and MARVIN. *Heart*, 1927, **14**, 27.
† DALE and GADDUM. *J. Physiol.*, 1930, **70**, 109.
‡ RANSON and BILLINGSLEY. *Amer. J. Physiol.*, 1916, **41**, 85.
§ SCOTT and ROBERTS, *J. Physiol.*, 1923, **58**, 168 SCOTT, *J. Physiol.*, 1924, **59**, 443.

These changes depend partly on the stimulation of the vasomotor and cardio-inhibitory centres by the venous blood, and partly on an action upon the heart itself.* We will first consider them with both vagi cut. For several seconds after leaving off the artificial respiration the only change noticed is the absence of the respiratory oscillations. Then the blood pressure suddenly rises rapidly (Fig. 435) and in another ten seconds may reach a height twice as great as it was previously. The heart frequency is almost unaltered. The blood pressure remains at this height for about a

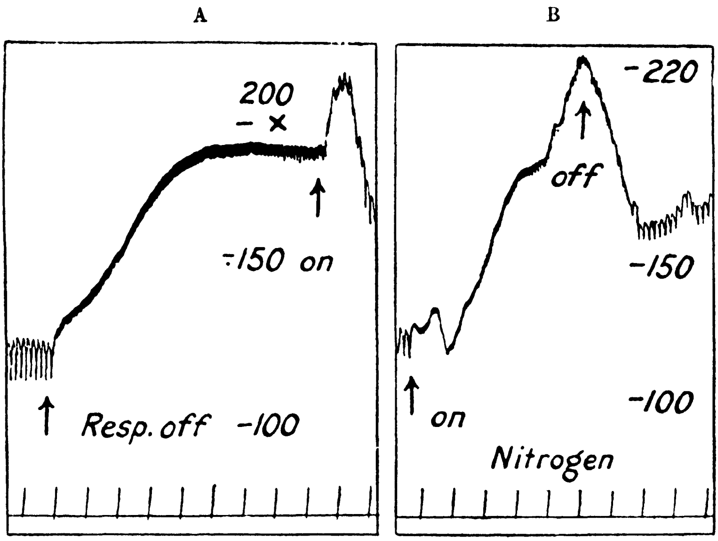


FIG. 435. Blood Pressure Changes in a Cat. (MATHISON.)
A. After cessation of respiratory movements. B. As a result of artificial respiration with nitrogen.

minute and then gradually falls, the heart beats becoming smaller and smaller until the pressure has sunk almost to zero. *This fall in pressure is due to the failure of the heart.* The heart gets overfilled, heart block ensues, and it gradually fails. The vessels, however, remain constricted until the death of the animal. This is shown by two facts. If, while the pressure is sinking, artificial respiration be recommenced, the heart, again supplied with oxygen, at once begins to beat more forcibly, and the blood pressure may rise to an even greater height than immediately after the commencement of the asphyxia. Again, if the volume of the kidney be recorded by means of the oncometer, the rise of general blood pressure produced by asphyxia is seen to be accompanied by a rapid shrinking of the kidney, and this shrinking endures until the animal dies.

Similar results are obtained when the vessels to the brain are ligatured.

We have spoken above of the phenomena of asphyxia as being due to the circulation of venous blood. This, however, comprises two factors, viz., a diminished percentage of oxygen and an increased percentage of carbon dioxide; and the question arises whether one or both of these factors are concerned. It is easy to investigate the action of each separately. A pure oxygen lack may be brought about by allowing an animal

* MATHISON. *J. Physiol.*, 1910, **41**, 416; 1911, **42**, 283.

to breathe some inert gas, such as nitrogen, or by injecting small amounts of cyanide into the blood ;* while the effects of accumulation of carbon dioxide in the blood and tissues may be produced by the administration of mixtures containing excess of oxygen, with varying percentages of carbon dioxide. In the first case, not only will the oxygen supply be reduced, but the tension of the carbon dioxide in the blood will also be kept below normal ; in the second case, while the CO_2 tension is raised, the tension of oxygen in the blood will be kept above normal. Mathison found that both conditions may concur in the production of the rise of blood pressure in asphyxia as shown by Figs. 435 and 436. There are certain minor details in these curves which are of interest. When the oxygen of the lungs is rapidly washed out with a neutral gas, the rise comes on about half a minute later than it would with pure asphyxia. In the latter case it seems that the first rise is due to the accumulation of carbon dioxide. The rise under nitrogen, however, when it occurs, is extremely abrupt, and the subsequent fall of blood pressure, *i.e.* the heart failure, is earlier in onset and more rapid than with ordinary asphyxia.

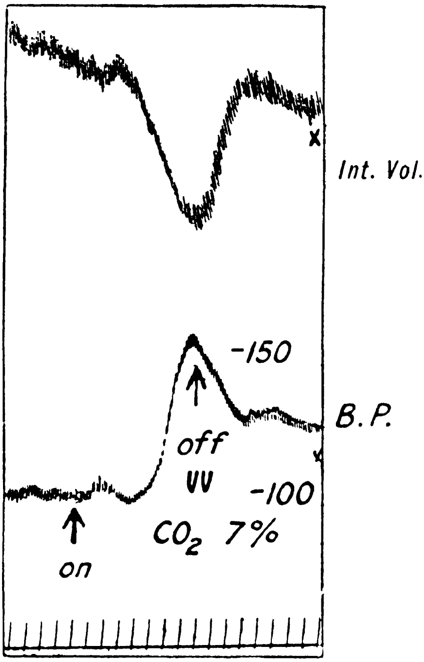


FIG. 436. Tracing of Arterial Blood Pressure and of Intestinal Volume, to show the Influence of a Moderate Increase in the CO_2 tension of the Blood. (MATHISON.)

When excess of carbon dioxide is administered, *i.e.* 5 to 10 per cent., a marked rise of pressure occurs which is almost entirely conditioned by stimulation of the vasomotor centres and resulting constriction of the peripheral arterioles. If a loop of intestine be placed in a plethysmograph, it will be seen that the rise of pressure coincides with a vascular constriction (Fig. 436). The rise of blood pressure due to the vascular constriction may be maintained for a considerable period since we do not get the failure of the heart that is observed in an ordinary asphyxia tracing. If partial oxygen lack, or abnormally increased tension of carbon dioxide, be continued for some time, a state of narcosis or paralysis finally ensues, which affects not only the higher centres but also those of the medulla, so that death may result without convulsions or excessive rise of blood pressure.

The action of cyanide resembles that of nitrogen breathing in its main features, but differs from it in having a more rapid onset, and a slower recovery. This is shown by Fig. 437, which also illustrates another feature of asphyxia. The blood pressure curve shows a second rise ; this is abolished if the suprarenals are removed : it is due to the liberation of adrenaline into the blood stream, which constantly happens in asphyxia.

* LOVATT EVANS. *J. Physiol.*, 1919, **53**, 17.

Is there any common factor in the two conditions of oxygen lack and carbon dioxide excess, which may account for the similarity in their effects ? It has been shown that, whenever there is a deficiency of oxygen, the metabolism of the tissues undergoes alteration, and lactic acid is formed. Lactic acid introduced into the blood stream also causes a rise of blood pressure similar to the asphyxial rise. When the cells of the vasomotor centre are deprived of oxygen, through lack of sufficient oxygenated blood,

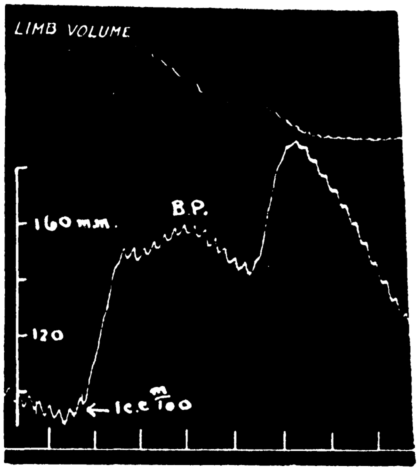


Fig. 437. Decerebrate Cat. Vagi divided. Upper tracing = limb volume. Second tracing = arterial pressure. Lower tracing = time (10 secs.). Injection of 1 c.c. of $\frac{m}{100}$ NaCN into femoral vein. (C.L.E.)

or are prevented from using it, as by cyanides, it is probable that they themselves produce lactic acid, which, like carbon dioxide, raises the H-ion concentration of their contents, and thus excites them.

If the vagi be left intact, the blood pressure tracing during asphyxia has quite another appearance. In this case, there is at first only a slight rise of pressure, but the heart begins to beat very slowly. This slow beat is due to the action of the vagus centre, and is at once abolished by section of the two vagi. The sparing of the heart by means of this vagus action enables it to last longer, so that heart failure comes on rather later than when the vagi are divided. In the increased vagus action, which occurs during asphyxia, two factors are probably involved. The cardio-inhibitory centre in the medulla partakes of the general excitation of the medullary centres, due in the first place to carbon dioxide excess, in the second to oxygen lack.

Rhythmic Activity. When the arterial pressure is high, especially in asphyxia, waves are often observed on the blood-pressure curve. These are of two kinds. In

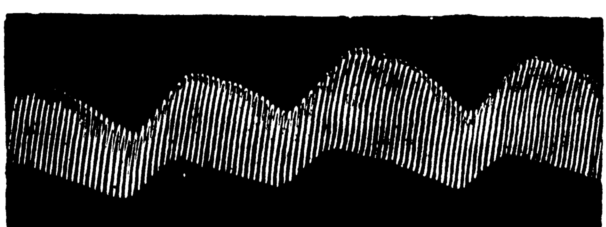


Fig. 438. Blood Pressure Tracing showing Mayer Waves. (C. J. Martin.)

completely curarised animals we may observe oscillations of blood pressure corresponding in frequency with the respiratory rhythm before curarisation. They are due to irradiation of impulses from the respiratory centre to the vasomotor centre. These

are the *Traube-Hering waves.** Besides these, others are occasionally seen which arise in a slow rhythmic variation of the constrictor impulses. These waves are known as the *Mayer waves* and are much slower in their rhythm than the respiratory waves. They are observed especially in dogs poisoned with morphia or after hæmorrhage. Fig. 438 represents such a tracing taken immediately after cessation of the artificial respiration, and shows only the heart beats and the Mayer curves. The presence of these waves may be ascribed to a state of rhythmically fluctuating excitability of the vasomotor centre. This excitation may arise in various ways. Often the rhythmicity is related to a rhythmically waxing and waning excitability of the respiratory centre.† A frequent cause of it is an increased venosity of the blood supplied to the centre.

Spinal Vasomotor Centres. The great fall of blood pressure observed after section of the cervical cord is not permanent. After a short time (especially in the cat), the pressure begins to rise, and if the animal be kept alive, may attain a height equal to that found in normal animals.

If the spinal cord be now destroyed, the blood pressure sinks to a very low level, because the animal has been, so to speak, bled into its own dilated blood vessels. Subordinated to the chief vasomotor centre in the medulla, there is a series of subsidiary centres in the grey matter of the lateral horns of the cord, whence arise the fibres which enter the white rami communicantes. By means of these spinal centres a certain degree of adaptation of the blood supply to the various parts of the trunk is possible in the spinal animal. The important co-ordination between the state of the blood vessels and the rate of the heart is, however, wanting, since the spinal centres are now cut off from the medullary centres. In the intact body we must regard these spinal centres as subordinated to the higher vasoconstrictor centre in the medulla, forming, indeed, a relay on the outward path from these to the

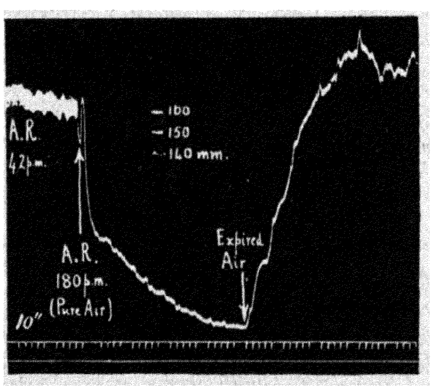


FIG. 439. Effect of Excessive Ventilation on Spinal Cat. Cord cut at second cervical level. With light artificial respiration (42 p. min.) the pressure is 160 m.m. Hg; this rapidly falls on ventilation at 180 p. min., but is restored by respiration, at 180 p. min., with expired air. (DALE and LOVATT EVANS.)

periphery; they are connected with the medullary vasomotor centre on each side by tracts of nerve fibres which descend in the lateral columns of the cord.

The spinal centres are also susceptible to changes in the composition of the blood supplied to them. If an animal be kept alive by means of gentle artificial respiration after division of the cord just below the medulla, the blood pressure soon resumes a normal level. If artificial respiration be now

* HERING. *Wiener Sitzungsber.*, 1869, **60**, 829; TRAUBE. *Z.f.d. med. Wiss.*, 1865, 881.

† AALKJÆR. *Skand. Arch. f. Physiol.*, 1935, **71**, 301.

discontinued the asphyxia excites the centres in the cord, and the pressure rises. Conversely, if the artificial ventilation be made excessive (acapnia), the blood pressure rapidly falls, but is speedily restored if ventilation, even at the same excessive rate, be carried on with air containing 5 per cent. carbon dioxide, or with expired air (Fig. 439).* After destruction of the spinal cord, these effects disappear. The spinal centres are also excited by lack of oxygen. There is a difference between the sensitivity of the spinal and medullary centres; the common factor is probably increased H-ion concentration, but the excitation threshold for the medullary centres is lower than that of the spinal centres.

THE PERIPHERAL TONE OF THE BLOOD VESSELS. Division of the sciatic and anterior crural nerves causes an immediate dilatation of the vessels of the lower limbs, in consequence of the loss of the tonic influence of the vasomotor centres. This dilatation passes off in a day or two, and the vessels resume a tone, *i.e.* remain in a state of average constriction, which can be increased or diminished by local conditions. This recovery of tone has been ascribed to the tonus of the muscular fibres themselves, aided perhaps by the presence of some substance in the blood.

VASOMOTOR REFLEXES

The vasomotor centre is constantly in receipt of impulses arriving both from the vascular system and from various other parts of the body. Hence reflex effects are produced by stimulation of various afferent nerves, and these may be classified, according to the area they affect, as general and local. If the reflex is sufficiently widespread, it causes a rise or fall of arterial pressure; such reflexes are distinguished as *pressor* or *depressor* reflexes respectively.

Vasoconstrictor Reflexes. A *pressor reflex* is one which induces a rise of general blood pressure by widespread constriction of the arterioles, especially in the splanchnic area. Practically all impulses, which, if consciousness were present, would be attended with pain or emotion, also cause vasoconstriction, which, if extensive and not otherwise compensated, causes a rise of arterial pressure. Thus a rise of pressure may be produced by the stimulation of almost any afferent nerve, including the central end of the splanchnic nerves.

These vasoconstrictor responses and the nervous pathways involved, have been elegantly demonstrated in man by Carmichael and his colleagues. One or more fingers or toes are placed in suitable plethysmographs and their volume curves are recorded. Stimulation of the skin, a loud noise, or the taking of a deep breath causes shrinkage of the digit by vasoconstriction.† After complete occlusion of the circulation in the arm by a sphygmomanometer armlet, the vasoconstrictor response still occurs in the fingers, showing that it is not a passive change. In cases of hemiplegia from lesions of the cerebral hemisphere the response in the affected side was normal.‡ Division of the whole nerve supply to the limb abolished the reflex, as did removal of the sympathetic supply alone (Fig. 440 A, B); division of the cerebrospinal supply without removal of the sympathetic supply left it unchanged (Fig. 440 C). Hence the path of the reflex in man is similar to that in the lower animals.

So far as the general arterial pressure is concerned, the most important impulses arriving at the centre are those from the vascular system itself,

* DALE and LOVATT EVANS. *J. Physiol.*, 1922, 56, 125.
† BOLTON, CARMICHAEL and STÜRUP. *J. Physiol.*, 1936, 86, 83.
‡ STÜRUP *et al.* *Brain* 1935, 58, 456.

especially from the heart and the carotid sinus, and those from the higher parts of the brain. Whatever the condition of the heart, the brain always demands a high arterial pressure. A failing heart therefore indirectly evokes constriction of the systemic vessels, a fact which may lead to a vicious

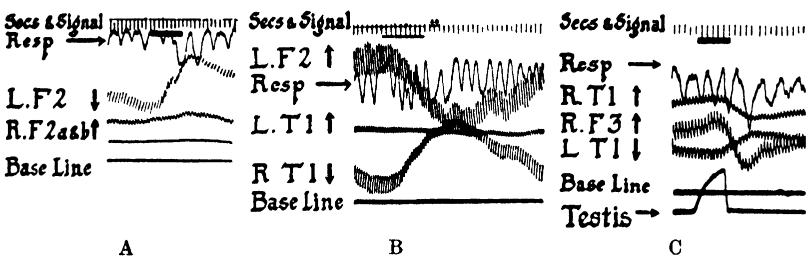


FIG. 440. Volume records of fingers or toes, and of respiration.

R.F., right finger; L.F., left finger, R T and L T., the right and left toes The arrows show direction of dilatation

A, lesion of right brachial plexus Left arm pinched No response of R F

B, left lumbar sympathectomy. Right arm pinched. No response in left toe

C, lesion of canda equina, motor and sensory paths to toes abolished, but sympathetic supply intact (from lower dorsal roots). Pressure on testis causes normal reflex. (STURUP, *et al*, 1935.)

circle in cases where the heart is unable to empty itself against the resistance of the blood vessels.

An important part is played by these reflexes in enabling the body to counteract the effects of severe hæmorrhage. This, by causing a fall of general arterial pressure, would set in action the pressor aspects of both the depressor and carotid sinus reflexes, thus causing vasoconstriction and cardio-acceleration, so that the fall of pressure would be much less than it would be without this regulating mechanism.

Another aspect is that which concerns the effect of posture on the circulation. It is well known that when we rise from the recumbent to the erect posture there is a compensatory vasoconstriction and cardio-acceleration, the effect of which is to prevent the arterial pressure from suddenly falling, especially in the head vessels. We can suppose that, on standing on the head, the reverse happens. These phenomena must play a large part in regulating the arterial pressure in the cerebral arteries of a long-necked animal, such as a giraffe, in which the fluctuation in hydrostatic pressure, if not so corrected, would be of the order of at least 150 mm. Hg. when the head was moved from the ground to its highest position.

Vasodilator Reflexes. The chief examples of *depressor* nerves, viz. the depressor or aortic nerve itself, and the nerve of the carotid sinus, we have already studied in dealing with the heart reflexes. The carotid sinus apparatus is probably at least as important as the aortic depressor nerve, with which it normally collaborates. By their co-operation a rise of arterial pressure excites a depressor reflex, with cardiac slowing, thus reducing or checking the rise. The reflex fall of pressure produced by stimulation of the central end of the depressor nerve is effected chiefly by dilatation of the splanchnic area (Fig. 441), though, as Bayliss showed, practically all the vessels of the body partake in the relaxation.

Vasodilator reflexes arising from the carotid sinus and aortic zones may be reinforced by similar, but minor, reflexes arising from other vaso-sensitive

zones, especially in the areas supplied by the cœliac and superior mesenteric arteries.*

Stimulation of the central end of the vagus, after section of both vagi, usually gives a depressor reflex because the nerve contains depressor fibres; but in some cases this may be preceded, or even replaced, by a rise of arterial pressure. This *vago-pressor* reflex appears to be normally in action when the blood pressure has been lowered by hæmorrhage; in such conditions section of the vagi produces a further fall of arterial pressure (McDowall).†

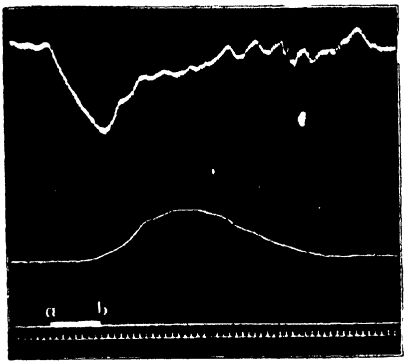


FIG. 441. Simultaneous Tracing of Arterial Blood Pressure and Splenic Volume from a Rabbit, showing the marked swelling of the spleen associated with fall of general blood pressure, on stimulation of the central end of the depressor nerve. The nerve was excited between *a* and *b*. (BAYLISS.)

According to Lewis ‡ most ordinary fainting attacks are of the nature of depressor reflexes, such as are mediated through the carotid sinus. The heart is slowed, and there is a fall of blood pressure. But even if the reflex cardiac slowing is prevented by administration of atropine, the fall of arterial pressure, and syncope, may still occur. The condition is probably due to exceptional excitability of the depressor reflex, so that stimuli of very various types may set it in action (vaso-vagal syndrome).

COMPENSATORY ADJUSTMENTS. Very commonly a vasoconstrictor reflex in one part of the body is accompanied by a vasodilator reflex in other areas, so that the arterial pressure may show no change, or may even fall. Under normal circumstances, every part of the body receives just enough blood for its metabolic requirements. Hence, activity must be associated with an increased flow of blood through the part. Two mechanisms are involved in this adaptation. In the first place, there is reflex dilatation of blood vessels in the part concerned, and in the second place, constriction of the blood vessels in the rest of the body, so that a normal or raised blood pressure is available for driving an increased supply of blood through the dilated vessels of the part. Thus, if both hind limbs of an animal be placed in a plethysmograph, it will be seen that stimulation of the central end of the anterior crural or peroneal nerve in the left leg causes dilatation of this leg and constriction of the leg of the other side.§ At rest, the organs of the chest and abdomen contain more than half of the total quantity of blood in the body, so that very little change in the capacity of these organs suffices

* HEYMANS, BOUCKAERT, FURBER and HSU. *Amer. J. Physiol.*, 1936, **117**, 619.
† McDOWALL. *J. Physiol.*, 1924, **59**, 41.
‡ LEWIS. *B. M. J.*, 1932, p. 873 (14, 5).
§ LOVÉN. *Ber. sächs. Ges.*, 1866, **18**, 85.

to furnish the extra supply of blood needed by any part during a state of increased activity.

The local axon reflexes, referred to above, also play an important part in bringing about adjustments of the blood flow in a part, and it has been shown by Fleisch that the reflex vasodilatation associated with activity of a tissue is in part a reflex due to chemical stimuli, as will be explained later. In the control of the blood supply to the tissues, the constriction and relaxation of the capillaries also probably play a large and special part.

These regulating reflexes provide yet another example, if any be needed, of one of the most fundamental of biological laws, namely, the law of the maintenance of constant conditions in the internal environment of the tissues, whereby a small change in the conditions gives rise to reactions which oppose further change, and oppose it with a force which increases as the change to be opposed augments.

INTRACRANIAL PRESSURE. The head regulates its blood supply according to its needs. If the head be supplied with blood by means of a circulation

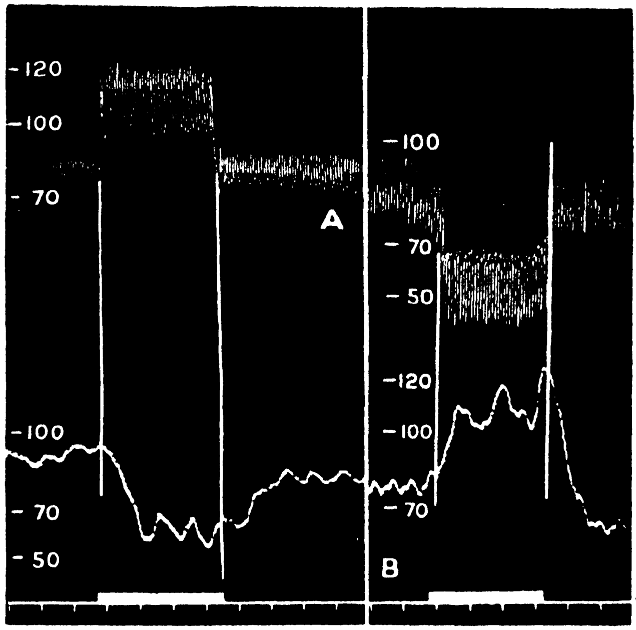


FIG. 442. Blood Pressure Tracings to show Influence of Changes in the Circulation through the Head on the Activity of the Vasomotor Centre.

The head was supplied with blood from a separate heart-lung preparation. The upper curve is the pressure in the head arteries which is controlled by the experimenter. The lower curve is the pressure in the vessels of the trunk. In A, a rise of pressure in the head causes vasodilatation and a fall of pressure in the trunk. In B, a fall of pressure in the head arteries has the reverse effect on the animal's own circulation. (ANREP and STARLING.)

distinct from that of the rest of the body, it is found that any rise of pressure in the arteries of the head, and consequent increased blood supply, brings about the inverse change in the arteries of the rest of the body—so that rise of pressure in the head vessels means a fall of pressure in other parts of the body, and *vice versâ* (Fig. 442).*

* ANREP and STARLING. *Proc. Roy. Soc.*, 1924, **97B**, 463.

This phenomenon, like the reflex cardio-inhibition which accompanies it, is a reflex from the *carotid sinus*, akin to the effects mediated by stimulation of the cardio-aortic nerves (*v.* p. 622),* and is not a consequence of the effect of pressure on the brain itself. The effect is reduced or abolished by denervation of the carotid sinuses.

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THE CHEMICAL REGULATION OF THE ARTERIOLES

METABOLITES. Another factor involved in the production of the increased blood flow through active organs, is a chemical stimulation of the vessels themselves, by means of substances (metabolites) produced as a result of

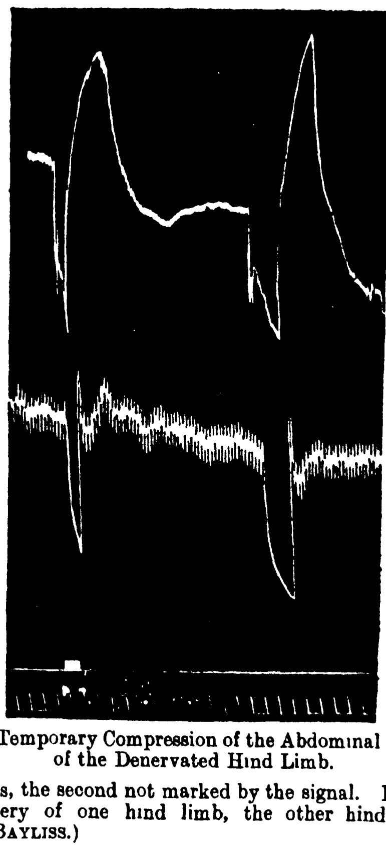

FIG. 443. Effect of Temporary Compression of the Abdominal Aorta on the Volume of the Denervated Hind Limb.

Two compressions, the second not marked by the signal. Blood pressure taken in the femoral artery of one hind limb, the other hind limb being in the plethysmograph. (BAYLISS.)

* FLOREY, MARVIN and DRURY. *J. Physiol.*, 1928, **75**, 209 ; HEYMANS, *Le sinus carotidien*, 1929.

activity. These substances are continually being produced, though in smaller amount, by the resting tissues, and when the blood supply is stopped for a time, they accumulate. If the blood is readmitted to the tissue, its vessels widely dilate—a condition called *reactive hyperæmia.* This is illustrated by Fig. 443. The great increase in the flow through the muscles, which accompanies muscular exercise, is probably brought about largely by similar means.

The exact nature of the metabolites concerned is still uncertain. It has been shown that the passage of blood containing lactic acid or carbon dioxide (both results of muscular metabolism) causes some dilatation of the blood vessels of a limb, but the dilatation which occurs in activity is greater than could be accounted for by the presence of these substances. A great variety of other metabolites have been found by Fleisch * to exert a dilator effect on the arterioles, though the same objection holds good as in the former instances. There are strong indications that it may be acetyl choline, or some closely related substance, which is known powerfully to relax the arterioles. It seems fairly certain that when a vasodilator nerve or an antidromic sensory nerve is stimulated, there is a liberation, in the region of its distribution, of acetyl choline. Another possible metabolite with vasodilator action is adenylic acid or adenyl pyrophosphate.

It might reasonably be asked, however, with respect to all these metabolites, why the arterioles should be affected by a substance which enters the blood down-stream from them, in the capillaries. The explanation, according to Fleisch † is that an ascending local reflex is effected through the nerves in the vessel walls. These nerves may be supposed to be stimulated by the various metabolites, and by a local reflex to cause arterial dilatation.

Action of Adrenaline. This substance, produced by the suprarenal glands, has a powerful influence on the calibre of the blood vessels. If 1 c.c. of a 1 in 10,000 solution of this substance be injected into the jugular vein, there is within a minute a widespread constriction of the arterioles, with the exception of those of the heart and brain. The same results occur after the vasomotor nerves have been divided, or the brain and spinal cord destroyed, so that there is no doubt that adrenaline acts directly on the blood-vessel wall. Owing to the large rise in arterial pressure, the action is therefore largely to augment the flow of blood through any organ the vessels of which are in a dilated condition, *e.g.* in an active muscle. When the splanchnic nerve is stimulated, there is an increased production of adrenaline, and the rise of arterial pressure shows a stepped curve, the first rise being due to the direct action of the vasoconstrictor nerves on the blood vessels, the second being brought about by the stimulation of the suprarenals and the discharge of adrenaline into the general circulation (Fig. 444). This is often associated with secondary cardiac effects due to the action of adrenaline on the heart. Similarly in Fig. 445 is shown the effect of brief stimulation of the splanchnic nerve on the blood pressure and on the volume of the limb of the cat. It will be noticed that the volume of the hind limb first increases passively with the rise of pressure, and then diminishes to much below its previous volume. This diminution is due to the discharge of adrenaline into the blood stream and is absent if the suprarenals have been previously destroyed. During asphyxia, the rise of arterial pressure is associated with the discharge of adrenaline. Similarly, when any pressor reflex is set up, as, *e.g.* by a

* Fleisch. *Arch. Internat. de Physiol.*, 1935, **41**, 141.
† Fleisch. *Arch. Internat. de Physiol.*, 1935, **41**, 141.

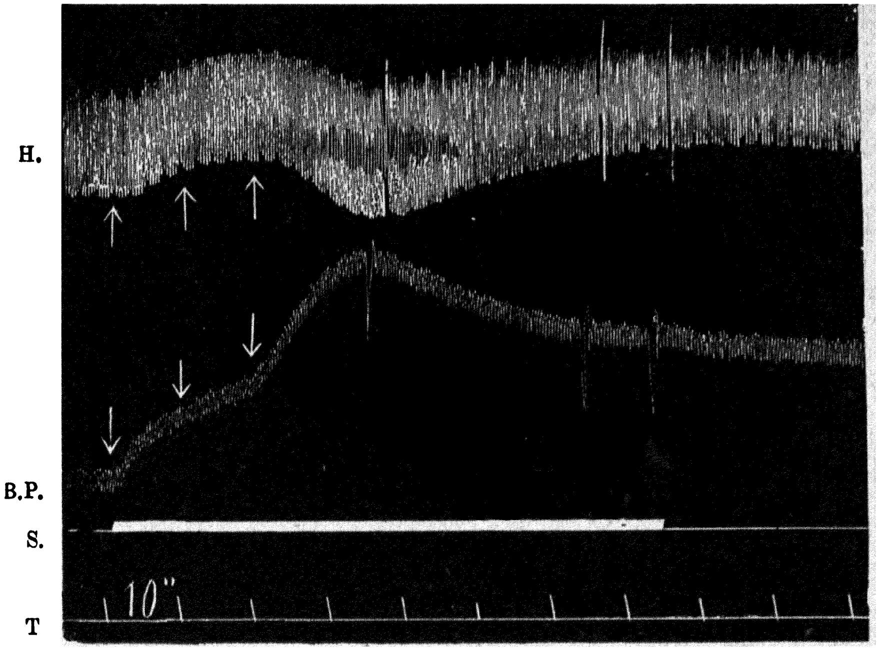

Fig. 444. Effect of Splanchnic Stimulation on Arterial Pressure and on Heart Volume.

H. Heart volume. (Systole causes a downward movement of the lever.) B P. Arterial blood pressure. S. Stimulation of splanchnic nerve. T. Time, 10 secs.

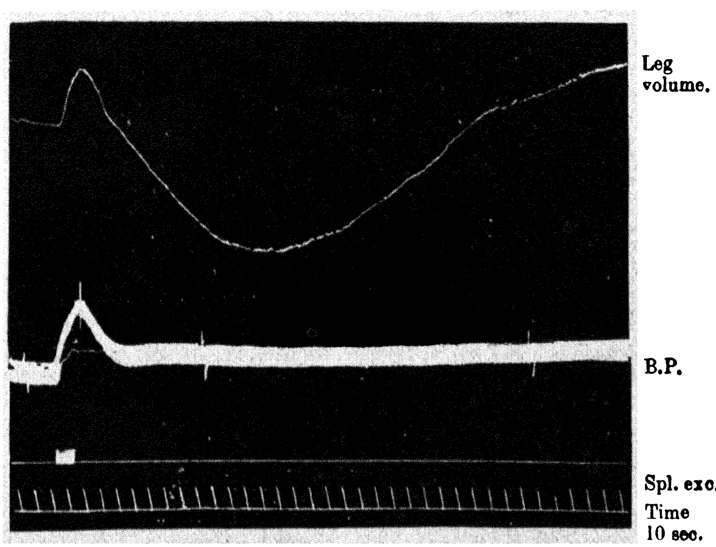

Fig. 445. Effect of Excitation of Splanchnic Nerves on the Blood Pressure and on the Volume of the Denervated Hind Limb of the Cat. (Bayliss.)

reduction of tension in the carotid sinus, it is accompanied by an increased discharge of adrenaline into the circulation.

The most intense action of adrenaline is on the skin vessels. The arterioles of muscle are dilated, and those in the small intestine only feebly constricted by adrenaline, while the coronary vessels of the heart are intensely dilated by it.

THE REGULATION OF THE BLOOD FLOW THROUGH THE CAPILLARIES

Up to the present, we have emphasised only two factors as regulating the blood flow through the peripheral parts of the body, viz. the arterial blood pressure, and the state of contraction of the arterioles supplying those parts. We cannot, however, regard the capillaries as merely inert channels. There is no doubt that both the calibre of, and the resistance to the flow of blood through, the capillaries are determined to a great extent by changes in their own walls; these changes consist in a swelling or shrinkage of the nuclei of the endothelial cells whereby the lumen of the capillary is narrowed or widened.*

When a tissue is in a state of rest, many of its capillaries close down completely, and so reduce its blood supply to a level commensurate with its small oxygen requirement. On activity, as the oxygen needs of the tissue increase, more and more capillaries open up, so that in a tissue like muscle there may be at least a hundred times as many capillaries in use as there were in the resting state.

Capillaries can be caused to contract by direct stimuli, or through the action of nerves, or by alteration in their chemical surroundings.

SKIN REACTIONS. The independent contractility of the minute vessels is well illustrated in the human skin, the colour of which is determined chiefly by the number and size of its minute vessels (' capillaries ').

If a blunt point be drawn over the skin, a mark, or *tache*, is left behind. On gentle stroking or on applying pressure with the finger the *tache* is white, and appears in fifteen to twenty seconds, being preceded by transient flushing. When the point is drawn over the skin with a firmer pressure, there is first a light flush (capillary dilatation), followed in fifteen seconds or so by (*a*) a much deeper flush along the actual track of the instrument (wide dilatation of capillaries and other vessels), and (*b*) at either side of the red *tache* so produced there appears a white *tache* (capillary constriction). These fade in from three to eight minutes. Microscopic inspection shows that the capillaries and venules in the pale area, though constricted, are in connection with vessels filled with blood, and the emptiness of these vessels can only be due to the fact that they have actively contracted against the pressure of blood with which they are supplied.

An experiment by Lewis, called the occlusion test, conclusively demonstrates capillary contractility. A Riva-Rocci armlet is placed in position and *rapidly* inflated to a pressure greater than the arterial pressure, *e.g.* 200 mm. Hg. The circulation in the arm is thus suddenly stopped, and the pressure in arteries, capillaries and veins then becomes equal; as the inflation was rapid, this pressure, though higher than the usual capillary pressure, is not very great. On now applying the skin reaction test, the white *tache* will be found to develop in the usual manner. Now, since the colour of the skin is due to blood contained in its minute vessels, it is evident that in such a limb, with occluded vessels, a contraction of arterioles could not deprive the

* SANDERS, EBERT and FLOREY. *Q. J. Exp. Physiol.*, 1940, **30**, 281.

capillaries of their contained blood, as might be objected if the circulation were proceeding.

Lewis has shown that the red reaction consists of two distinct reactions—a localised one, due to relaxation of *all* the skin vessels, and a spreading flare, due to an axon-reflex, which causes relaxation of the skin arterioles, often for considerable distances, when powerful stimuli have been used.

In a certain number of subjects, the skin after the *tache* tests shows some urticaria, while in others a definite wheal results, owing to the production of local œdema—a condition called *dermatographism.* The *triple response,* i.e., local vasodilatation, spreading flare and local œdema, may be obtained by stimulation of the skin by mechanical means, heat, cold, electricity and by various chemical substances, especially histamine.

Whether these reactions characteristic of the minute vessels of the skin are properties of the capillaries in other regions is uncertain. It would be expected that the skin would be best adapted to show responses to mechanical stimulation, but there is a certain amount of evidence for similar responses in other parts to stimuli equally appropriate.

METABOLITES. The dilator effect of metabolites on the arterioles, which we have already studied, seems to be shared by the capillaries. In such a case, it is difficult to dissociate the effects of arterial dilatation from those of capillary dilatation. Acetyl choline, or substances akin to it, chiefly relaxes the arterioles. At least one chemical substance is known, however, which has diametrically opposite effects on the two sets of vessels, viz. *histamine.* This substance has been shown to have a constrictor effect on the arterioles, and a dilator effect on the capillaries.* Moreover, it so increases the permeability of the capillary walls, that fluid rapidly passes out from the blood stream into the tissues. It has been suggested that when tissues are subjected to damage, histamine or something resembling it (H-substance) is either liberated from or produced in the cells, and results in local capillary and venule dilatation as seen, for instance, in the skin reactions. If a minute amount of histamine is introduced beneath the skin by pricking through a drop of 1 : 1000 solution, a typical triple response is seen at the site.

WOUND SHOCK. It has been suggested that the excessive production of histamine, or of other substances with a similar action, plays an important part in giving rise to the symptoms of surgical, or wound, shock. In this condition, which is found notably after widespread laceration, and consequent destruction of the tissues, especially of the muscles, there is a continually increasing fall of the blood pressure, accompanied by an ever lessening volume of blood in circulation. This lowered blood volume is due to loss of plasma, and the blood has a high red cell content and is correspondingly viscous. Since the lowering of blood pressure does not depend on any direct action on the heart, nor on vasomotor paralysis, it has been concluded that the prime factor at work is a general dilatation of the capillaries, leading to stagnation of the blood in these vessels, and an increased exudation into the tissues, thus causing a constant leak of fluid from the general circulation.

The evidence that these changes are due to the liberation of histamine, or some closely-allied substance, is mainly circumstantial, though suggestive. There is, first, the fact that histamine can, on injection into the circulation, cause effects closely resembling those seen in surgical shock; or, when injected into the tissues, cause local changes essentially identical with those

* DALE and RICHARDS. *J. Physiol.*, 1918, 52, 110.

produced by local injury. There are reasons, however, for not accepting all this evidence at its face value.*

Secondly, there is the fact that histamine (and also choline esters) can be extracted from fresh tissues, particularly the lungs, in which, apparently, it is either present as such during life, or is produced when the cells are damaged. The depressor substances present in nearly all tissue extracts have been shown by Dale and Dudley to contain both histamine and esters of choline; either substance produces a fall of blood pressure.

Since histamine appears to be frequently produced in active or damaged localities, and to be capable, when present in sufficient amount, of producing general effects, there must be some means by which its accumulation is normally prevented or counteracted. It was shown by Best and McHenry † that this is possible owing to the presence of an enzyme, *histaminase*, which is present in many tissues, but especially in kidney and intestine. These organs rapidly destroy any histamine present in blood passing through their vessels.

The chemical explanation of shock has not held its ground, and it is now clear that it can only in part explain the condition. There is no evidence that the histamine content of the blood from a traumatised part, or of that in general circulation in shock patients, is much, if at all, increased. It has been claimed that part of the phenomenon is of reflex origin, and that if the nerves from the damaged tissues are divided, or blocked by novocaine, shock is reduced or lacking. Another suggestion is that it is due to exhaustion of the adrenal medulla, and yet another that the lack of cortical adrenal hormone may be a factor.

Action of Nerves. The capillaries are abundantly supplied with nerve fibres, and capillary constriction has been observed under the microscope on stimulation of the sympathetic nerve supply.‡ There is also a connection between nerve lesions and the calibre of the capillaries in the area supplied by the nerves. Thus, if in the cat the sciatic nerve be cut on the right side, for the next few hours the pad of the foot on that side is flushed and warmer than the left foot. The next day the flush has disappeared, in fact the pad of the right foot may be paler than that of the left foot. The right foot is, however, still a degree or two warmer than the left. This condition may be explained on the assumption that the immediate effect of cutting the sciatic nerve is to cause dilatation both of the arterioles and of the capillaries. The capillary dilatation passes off, so that on the day after the section, although the arterioles are still dilated and there is a more rapid flow of blood through the pad, the capillaries are contracted, so that the pad is paler than on the opposite side.

Whether there are dilator nerves to the capillaries is uncertain, but some claim that axon reflexes are demonstrable, similar to those affecting arterioles.

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* Grant and Duckett, *Heart*, 1929, **14**, 337; Rous and Gilding, *J. Exp. Med.*, 1930 **51**, 27; Percival and Scott, *J. Pharm.*, 1931, **41**, 147.

† Best and McHenry. *J. Physiol.*, 1930, **70**, 349.

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CHAPTER XXXV

THE PULMONARY, HEPATIC AND CEREBRAL CIRCULATIONS

THE PULMONARY CIRCULATION

In the lungs there is an extensive system of wide arterioles and capillaries derived from the pulmonary artery, and presenting very little resistance to the flow of blood, and also a much smaller supply from the aorta by way of the bronchial arteries. There is a certain amount of anastomosis between the pulmonary and bronchial circulations, though mainly only in the smaller vessels, so that probably but little bronchial blood reaches the alveolar capillaries.* The mean pressure in the pulmonary artery probably does not exceed 15 to 20 mm. Hg., *i.e.* about one-sixth of the mean aortic pressure.

The capillaries of the lungs may vary passively in size, according to the conditions. Thus, whereas at the height of inspiration the blood contained in the lungs is about one-twelfth of the whole blood in the body, this amount is said to be diminished during expiration to between one-fifteenth and one-eighteenth, and by forcible artificial inflation of the lungs may be lessened to one-sixtieth. Such changes may exercise a considerable effect on the systemic blood pressure. Again, if the circulation rate is increased, the whole of the pulmonary vascular bed will be distended, and as much as one-fifth of the blood may be in the lungs (Kuno †). On the other hand, the distensibility of the lung capillaries may play an important part in enabling the lungs to act, so to speak, as a reservoir for the left side of the heart. If there is a temporary excess of output on the right side that cannot be dealt with at once by the left heart, the excess is taken up for a time in the lung capillaries.

The mean pressure in the pulmonary artery is, as stated above, only of the order of 20 mm. Hg. It depends on the output of the right ventricle and the resistance in the lungs, and of these the one most subject to variation is the former.

The mean pressure in the pulmonary veins is very low, being at or about zero, and somewhat greater than that in the left auricle. The gradient between pulmonary artery and pulmonary vein is thus a steep one, as would be expected in view of the high velocity of the blood in the pulmonary circuit. Although an increased output of the right heart leads to a definite rise of pressure in the pulmonary artery, the pressure in the pulmonary veins is little increased. This fact, which is because the left auricle is normally competent to keep the pulmonary veins adequately drained, results in further steepening of the gradient of pressure which drives blood through the lungs.

An important question is raised as to the influence of changes of aortic pressure, due to alteration of systemic resistance, on the pressures in the pulmonary circuit. Experiments on the heart-lung preparation indicate that a rise in the aortic pressure, while the venous inflow into the heart is

* Berry and Daly. *Proc. Roy. Soc.*, 1931, **109B**, 319.

† Kuno. *J. Physiol.*, 1917, **51**, 154.

kept constant, is accompanied by a corresponding rise in the pulmonary artery pressure. This has been often ascribed to "back pressure," *i.e.* to a relative failure of the left heart to deal with the blood flowing into it. This cannot be the case, however, for the left auricular pressure is not raised (Fig. 446). The phenomenon is due to increased output of the right heart caused by increased flow through the coronary arteries induced by the rise of aortic pressure.* Similar conditions contribute to the parallelism between pulmonary and systemic arterial pressures in the intact animal. Only when the left ventricle is greatly dilated and the mitral valve incompetent, are there likely to be pronounced back pressure effects.

The pulmonary artery pressure shows considerable variations, about

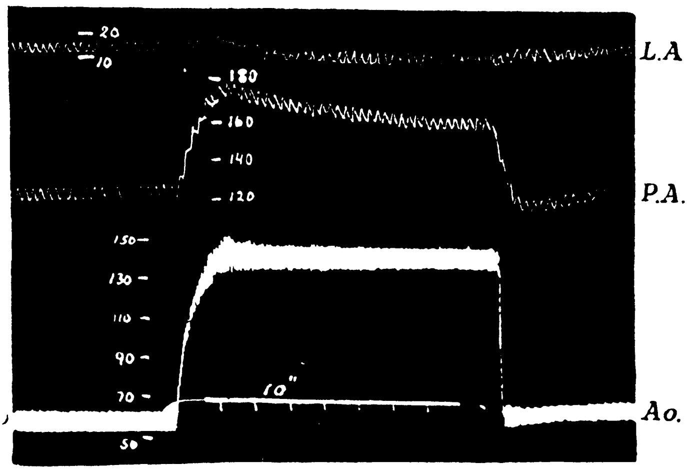

FIG. 446. Effect of rise of systemic pressure on the pressures in the left auricle and (m m. H_2O) pulmonary artery. Heart-lung preparation. (ANREP and BULATAO.) *

30 mm. Hg. with each heart beat, so that the pulse pressure is greater than the average pressure †; this means that the resistance in the pulmonary bed is low. The pulsations resemble those of the systemic arteries (Wiggers). There are also slower variations of pressure due to the respiratory movements; the pressure falls during inspiration and rises during expiration. In the pulmonary veins the pressures vary less with respiration, and probably in the reverse direction. These results are due to a lowering of resistance in the pulmonary capillaries during inspiration (Daly ‡).

The resistance of the pulmonary circuit can undoubtedly be varied by the action of both vaso-constrictor and vaso-dilator nerves.§ The constrictor fibres are probably of sympathetic origin, and derived from the upper thoracic nerves, from which they reach the thoracic and cervical vago-sympathetic nerves. Addition of adrenaline to the circulating blood causes pulmonary vaso-constriction with rise of pulmonary artery pressure. The dilator fibres are probably derived from the vagi. Stimulation of the vago-sympathetic may therefore cause either vaso-constriction or vaso-dilatation, according to which of the component fibres are most affected. Reflex pulmonary vaso-constriction has been claimed to result as a consequence of the stimulation of sensory nerve roots and of the central end of

* ANREP and BULATAO. *J. Physiol.*, 1925, **60**, 175.
† MATTHES and HOCHREIN. *Arch. f. Exp. Path.*, 1932, **167**, 678.
‡ DALY. *J. Physiol.*, 1930, **69**, 238.
§ DALY and v. EULER. *Proc. Roy. Soc.*, 1932, **110**, 92.

the splanchnics, and reflex dilatation from stimulation of the depressor nerve. It is uncertain to what extent the nervous control of the pulmonary bed is exercised under normal conditions.

Effect of Respiration on Systemic Blood Pressure. If we examine a tracing of the general arterial pressure, we notice that with each inspiration the blood pressure rises, and with each expiration it falls. The synchronism of the rise and fall with the respiratory movements is not exact, and varies with the rate of the respiratory movements, since the rise continues for a short time after the beginning of expiration, and the fall continues into the beginning of the next inspiration, so that in slow breathing the highest point of the curve occurs at the beginning of expiration and the lowest point at the beginning of inspiration. During the rise which accompanies inspiration, the heart beats often become accelerated. This is due to a "Bainbridge reflex," the acceleration being caused by the increased filling of the right heart by aspiration of blood into it during inspiration, as explained below. On dividing both vagi, this difference in the pulse rate during inspiration and expiration disappears, but the main features of the blood pressure curve remain; when respiration is artificially produced by forcing air into the lungs, the effect of the movements is reversed, *i.e.*, the rise now occurs during the expiratory phase; these various facts indicate that we must look for some mechanical explanation of the respiratory undulations.

As we shall see later, under normal conditions the lungs are in a state of stretch, and in consequence of this condition they are constantly exerting a pull on the chest wall. As soon as we admit air into the pleural cavity, the lungs collapse. As the chest expands in inspiration it drags the lungs still more open, so that their pull on the chest wall becomes greater, and hence the negative pressure in the pleura is much increased during forcible inspiration. The heart, great veins and arteries in the thorax are separated from the pleural cavity only by a thin membrane, so that they are practically exposed to any pressure which may exist in the pleural cavity, whereas outside the thorax all the vessels are exposed to a positive pressure.

Any fluid will always flow from a point of higher to a point of lower pressure. Hence, when the thorax enlarges during inspiration, there must be an aspiration of blood into it from peripheral parts. This aspiratory force will not influence arteries and veins alike. The arteries, having thick, comparatively non-distensible walls, will be very little affected by the increase of negative pressure in the thoracic cavity, whereas the thin-walled distensible veins, auricles and right ventricle, will be largely influenced. The total result, then, of the increasing negative pressure in the pleural cavities, is to increase the flow of blood from the veins into the heart, especially on the right side, without affecting to any appreciable degree the outflow of blood from the heart into the arteries. The more pronounced the fall of pressure in the thorax, the greater will be the amount of blood sucked into the heart from the veins. *During* inspiration, therefore, the inflow into and output from the right heart will be more rapid than during expiration, and this factor in itself will tend to raise the arterial blood pressure. The inspiratory descent of the diaphragm will, moreover, tend to increase the inflow into the heart by raising the positive pressure in the abdomen, so that the blood is pressed out of the abdominal veins, and sucked into the heart and the thoracic veins. During expiration, all these effects are reversed.

Although extreme expansion of the lungs increases the resistance to the flow through their vessels, presumably by lengthening and narrowing the latter, expansion of the lungs in normal inspiration considerably distends the vessels, and so increases the capacity and lowers the resistance of the pul-

monary circuit.* The increased circulation rate produced by inspiration also distends the vascular bed. When the thorax expands, the immediate effect of the pull on the vessels that have thin walls is to fill them up with blood, and this extra blood being diverted from its onward movement leads at first to a reduced flow into the left ventricle. Hence, at the onset of inspiration, the systemic blood pressure falls even further than during expiration. For the converse reason that the expanded vessels partly empty at expiration, and add the blood so expelled to the pulmonary current, the systemic pressure at the commencement of expiration continues still further the rise established during inspiration. Lewis † regards the pericardial pressure, *i.e.* the direct influence of the thoracic movements on the heart, as playing a much more important part than changes in the pulmonary circulation in the production of the respiratory undulations in the blood pressure. He showed, moreover, that, in man, the effect of respiration on arterial blood pressure may vary according to the type of respiratory movement, a deep costal inspiration (not prolonged) causing a pure fall, while a deep diaphragmatic inspiration gives a pure rise of blood pressure. In expiration the reverse effects hold. He concludes that, in man, it is not possible to make any *general* statement as to the nature of the blood pressure response to a particular respiratory act.

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THE HEPATIC CIRCULATION

The blood flow through the liver is very large—probably about half the total flow along the inferior vena cava is contributed from this source. The liver is supplied by two main vessels, the hepatic artery and the portal vein, and the former probably contributes at least one-quarter of the blood flow and about 40 per cent. of the oxygen to the liver.‡

The liver, on section, is seen to be divided off into lobules composed of columns of polygonal cells, radiating from the centre. The portal vein, which drains the blood from the spleen and alimentary canal, breaks up into branches which lie at the periphery of the lobules, forming the interlobular veins, and send off numerous sinusoids which pass inwards between the columns of cells to join the intralobular vein lying at the centre of the lobule. From the intralobular vein the blood passes by the large sublobular veins into the hepatic veins and inferior vena cava. In an injected specimen it is easy to see that every liver cell is adjacent to at least one blood capillary. The portal vein conveys only venous blood to the liver. The lymphatics run in Glisson's capsule.§

The hepatic artery principally supplies the connective tissue of Glisson's capsule, in which it breaks up into capillaries, and these are drained partly into venules which join the portal vein branches and partly into the sinusoids near to the margins of the liver lobules,|| so that the whole of the venous blood derived from the hepatic artery supply mingles with the general portal blood stream.

Complete loss of its arterial supply leads to necrosis of the liver, ¶ though owing to the existence of anastomotic connections between the hepatic and

* DALY, *Journ. Physiol.*, 1928, **65**, 422; HEINBECKER, *Am. J. Physiol.*, 1927, **81**, 170.

† LEWIS. *J. Physiol.*, 1908, **37**, 213, 233.

‡ BURTON-OPITZ, *Q. J. Exp. Phys.*, 1911, **4**, 113; GRAB, JANSSEN and REIN, *Z. f. Biol.*, 1929, **89**, 324; SCHWIEGK, *Arch. f. exp. Path. Pharm.*, 1932, **168**, 693.

§ LEE. *Contrib. to Embryol.*, 1923, **15**, 63.

|| OLDS and STAFFORD. *Bull. Johns Hopk. Hosp.*, 1930, **47**, 176.

¶ BAINBRIDGE and LEATHES, *Biochem. J.*, 1907, **2**, 25, CAMERON and MAYES *J. Path. and Bact.*, 1930, **33**, 799.

diaphragmatic arteries, occlusion of the main hepatic artery may not be incompatible with life, even in man.

Complete and sudden obstruction of the portal vein leads to speedy death, with engorgement of the abdominal viscera which drain into the portal vein, while the occlusion of one of its branches leads to atrophy of the lobes of liver supplied by it.

The resistance to the flow of blood through the liver is normally very slight, as is shown by the fact that the pressure in the portal vein, which drives so large a blood flow through the liver, is only about 8 mm. Hg. The pressure shows respiratory fluctuations, rising at inspiration, owing to a massaging action of the rhythmically descending diaphragm.

When there is a permanent increase of the resistance, as happens in diseases attended by portal cirrhosis, the blood flow along the portal vein finds its way into the inferior vena cava partly through a short circuiting of the liver by the enlargement of anastomotic connections which normally are insignificant, *e.g.* between the gastric and the œsophageal veins, the inferior mesenteric and the hæmorrhoidal branches of the internal iliac veins, or between the coronary veins of the stomach and branches of the phrenic veins: the spleen becomes enlarged and the surface of the abdomen is covered with the enlarged veins subserving the anastomosis. It is possible to create such a condition experimentally in animals by increasing the pressure in the portal vein,* and use has been made of this fact for extirpating the livers of animals for experimental purposes.

It is impossible to remove the liver directly in mammals, since ligature of the portal vein, which would be a necessary step in the operation, causes the blood to be dammed up behind the ligature in the portal area. The intestinal wall gets full of effused blood, and the animal dies within a few hours, being bled to death, so to speak, into its portal vessels. A way of partially obviating this difficulty was suggested by Eck.† Before ligature of the portal vein, this vessel was joined side by side to the vena cava, so that after the ligature the portal blood could flow directly into the general circulation without passing through the liver (Eck fistula). The liver was not removed, and a path to it was still open by the hepatic artery. As liver extirpations, the experiments were inconclusive, because the liver was still present and provided with a large, though abnormal circulation. The operation still has its uses, however, in cases where it is desired to short circuit the portal circulation.

More satisfactory extirpations were performed by Mann and Magath ‡ and their collaborators. The operation is performed in three stages. First an operation like an Eck fistula is performed, except that after connecting the portal vein laterally with the vena cava, the vena cava and not the portal vein, is tied above the anastomosis. All the blood from the vena cava below the liver is thus diverted into the portal vein. After recovery from this it is found that owing to the increased pressure in the portal vein a bye-pass circulation is soon established between the portal vein and thoracic veins, *e.g.* the internal mammary and azygos. When this has occurred, the second operation, viz. ligature of the portal vein at the portal fissure, is performed. Now all the portal blood flows by the collaterals to reach the inferior vena cava above the liver. Lastly, the hepatic artery is ligatured and the liver removed, together with the length of inferior cava into which its vessels drained.

The portal area also plays an important part in the circulation by acting as a variable reservoir for blood.§ Not only is the volume of blood which passes the liver great, but the channels through which it is conducted are wide, so that the amount of blood present in the liver at any moment is considerable. The portal vein, with its rootlets (including the spleen), represents a reservoir intercalated between two resistances, viz. the arterioles of the splanchnic area centrally and the smaller liver vessels peripherally.

* CL. BERNARD. "Le diabète," 1877, p. 317. (Paris, Baillière.)

† JERUSALEM, *Zntrlbl. f. Physiol.*, 1910, **24**, 837 (method); DALE and LAIDLAW, *J. Physiol.*, 1919, **52**, 351 (simplified method).

‡ MANN, *Amer. J. Physiol.*, 1921, **55**, 285; MANN and MAGATH, *Ergebn. d. Physiol.*, 1924, **23**, 212 (simplified method); SOSKIN, *J. Lab. Clin. Med.*, 1931, **16**, 382.

§ KROGH. *Skand. Arch. f. Physiol.*, 1912, **27**, 227.

This reservoir when distended can hold about a third of the blood in the body. Filling of the portal reservoir can be effected by dilatation of the primary (splanchnic) resistance or by constriction of the secondary (hepatic) resistance, and conversely a partial emptying of the reservoir results from constriction of the primary and/or dilatation of the secondary resistance.

The regulation of the splanchnic arterioles, by which the rate of entry of blood into the portal vein is controlled, is by the usual vasoconstrictor nerves of the splanchnic area. On stimulation of the splanchnic nerves the portal vein pressure, which is usually low (about 8 to 10 mm. Hg), at first falls a little and then rises abruptly * to about double the normal value. The cause of the small and transitory initial fall is probably the immediate reduction of the flow of blood into the portal vein in consequence of constriction of the splanchnic arterioles. The subsequent rise is due to several causes, and is no doubt similar to the rise produced by adrenaline.† Part of it is due to constriction of the smallest branches of the portal vein within the liver. There is, however, another mechanism of considerable importance, viz. the communication, in the liver, between the hepatic artery and portal vein circulations. In consequence of this, the rise of general arterial pressure, which results when the splanchnics are stimulated, or when adrenaline is given, causes more blood to pass to the liver by the hepatic artery and so to raise the pressure in, and thus act as a resistance to the flow through, the vessels supplied by the portal vein.

That there is a connection between the hepatic artery and portal vein circulations is shown by the further fact that when the superior mesenteric artery or vein is tied off, the portal vein pressure, though it falls, does not fall to a low level. Clamping of the hepatic artery alone also causes a fall of portal pressure for the same reason. A further factor which causes an increase of portal pressure after the administration of adrenaline is that the moderate constriction of the mesenteric arterioles is afterwards overcome by the high arterial pressure,‡ so that more instead of less blood enters the portal vein.

When there is general vasoconstriction of the splanchnic area and of the liver vessels, as occurs after administrations of vasopressin, the portal pressure falls, owing to restriction of inflow, since the arterial pressure is unable to overcome the high resistance of the constricted vessels.

In some animals, notably the dog, it is found that on perfusion of the liver outside the body, the flow soon declines unless a very high pressure is used for the perfusion. This is because there is, at the outlet of the hepatic veins, a strong layer of plain muscle which can contract and so control the outflow from the liver. It is caused to contract by histamine and relaxed by adrenaline or sympathetic stimulation,§ and by oxytocin.‖ Under conditions of artificial perfusion, and in certain shock-like states, these sphincters are tightly contracted, with the result that the portal pressure rises and the liver rapidly swells.

THE CEREBRAL CIRCULATION

The grey matter of the brain is very richly supplied with blood vessels from the *pia mater*, and the brain receives about 90 to 180 c.c. blood per 100 g. per minute. Any interference with the blood flow through the brain

* BAYLISS and STARLING. *J. Physiol.*, 1894, **16**, 170; 1895, **17**, 120.
† MCMICHAEL. *J. Physiol.*, 1932, **75**, 241.
‡ CLARK. *J. Physiol.*, 1928, **66**, 274; 1930, **69**, 171.
§ BAUER, DALE, POULSSON and RICHARDS. *J. Physiol.*, 1932, **74**, 343.
‖ HOLTZ. *J. Physiol.*, 1932, **76**, 149.

rapidly checks its functions, because the brain tissues have a very rapid gaseous metabolism, at least 200 c.c. of O_2 per 100 g. brain per hour.*

The smaller arteries derived from the pial vessels penetrate into the substance of the brain and anastomose freely with one another. Capillaries are abundant, especially in the grey matter of the cerebral cortex, where they aggregate about 1 m. of capillary vessel per c.mm. of nerve tissue, a figure which, however, is low compared with, say, cardiac muscle. The white matter is less vascular and uses less oxygen than the grey.

In all higher animals, the brain is enclosed in a rigid bony cranium. In the child, before the cranial vault is fully ossified, part of this vault consists of membrane, the anterior fontanelle. The fontanelle pulsates with each heart beat, as well as with rise of venous pressure, such as that produced during strong expiratory efforts. When ossification is complete, such alterations in the volume of the cranial contents are impossible. When viewed through a skull window, little movement of the pial arteries is visible, apart from a straightening out at points where they change direction, at each heart beat. If any expansion of the arteries occurs, room for this expansion has to be found by contraction of some other part of the cranial contents. The rapidly variable contents of the cranium are the blood and the cerebrospinal fluid. We find that each arterial beat is associated with a corresponding compression of the veins, and if a cannula be introduced through the occipital bone into the torcular Herophili, the venous blood is seen to pulsate out with each arterial pulse. If there is a rise of arterial pressure, although the arteries may expand somewhat at the expense of the veins, there can be little dilatation of the whole organ. The first, and chief, effect of the rise of pressure will be to cause an increased pressure slope in the cranial vascular system, and therefore augmented velocity of flow through the system. As a result of a more prolonged rise of pressure a certain amount of dilatation of the vessels may ensue, but only at the expense of the cerebrospinal fluid. Since this is small in amount, any expansion of the brain due to vascular causes must be limited. The converse applies for shrinkage when the general arterial pressure is lowered.

BRAIN PRESSURE. If a trephine opening be made into the cranial vault, the brain bulges into the opening. By screwing a window into the trephine opening, we can find the pressure necessary to force the brain back to its previous position. This is known as the intracranial pressure; it is, as might be expected, approximately equal to the cerebrospinal pressure and to the pressure in the venous sinuses, and is closely dependent on the latter. Forced expiratory efforts may raise the pressure from 30 to 50 mm. Hg. In the vertical position, in man, the pressure may be slightly negative, in consequence of the tendency of the venous blood to run downwards towards the heart.

Regulation of the Blood Supply to the Brain. The free anastomoses in the circle of Willis ensures an adequate distribution of blood to all parts of the brain, even should one or more of the contributory arteries get accidentally occluded. The blood supply to the brain is primarily dependent on the arterial pressure, and this, as we have seen, is specially controlled by the carotid sinuses. Hence, any tendency for the carotid arterial pressure to fall is at once counteracted by systemic circulatory changes of such a nature as to tend to restore it to normal again. If this pressure fails, the functions of the brain are affected and loss of consciousness rapidly ensues. This is what occurs when a person, who is weak from long illness, faints on

* CHUTE and SMYTH. *Q. J. Exp. Physiol.*, 1939, **29**, 379.

suddenly getting up from bed. In the normal individual, the change in the circulation with alteration of bodily position, which would be produced by the action of gravity, is at once counteracted through the vasomotor system.

As a subsidiary means of regulation, the vessels of the *pia mater* are themselves supplied with vasomotor nerves. In order to demonstrate these it is necessary to observe the pial vessels through a window let into the skull, so as to ensure the normal rigid enclosure of the brain.* It can then be shown that stimulation of the central end of the cervical sympathetic nerve causes constriction of the pial vessels † (Fig. 447). Stimulation of the vagus depressive fibres causes reflex vasodilatation, the efferent vasodilator fibres apparently reaching the cerebral vessels from the VIIth nerve.

The action of drugs is on the whole similar to that on other vessels. Intravenous injection of small amounts of adrenaline may also cause con-

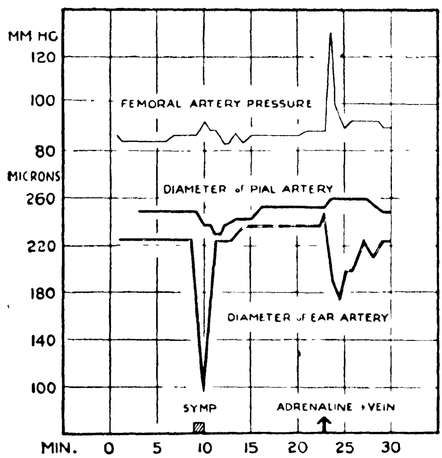

FIG 447 Showing diameters of pial artery and of a small ear artery in the cat, together with femoral artery pressures Observe that stimulation of the cervical sympathetic at " symp " causes much greater constriction of ear artery than of pial artery. Injection of 0·01 mg adrenaline intravenously causes constriction of ear artery, and a large rise of arterial pressure, on account of which the pial artery passively dilates. (FORBES, FIRNLEY and MASON)

striction, but often this is so weak that it gives way to passive expansion of the vessels owing to the rise of general blood pressure ‡ (Fig. 447). Acetyl choline causes dilatation of the vessels, though if the arterial pressure falls, there may be no increase of cerebral blood flow. Histamine gives similar results ; most anæsthetics also cause vasodilatation The action of pitressin is doubtful.

Under asphyxial conditions, local or general, there is dilatation of the cerebral vessels. Both oxygen lack and carbon dioxide excess act in this manner, but the action of carbon dioxide is the more potent, and is due to a direct effect on the vessel walls.

There is some evidence that the vessels of the brain are dilated during states of increased activity.

When the intracranial pressure is increased to a level above that of the arterial pressure, the flow of blood through the brain is stopped, and uncon-

* FORBES. *Arch. Neurol. and Psychiat.*, 1928, **19**, 751.
† FORBES and WOLF. *Ibid.*, 1928, **19**, 1057.
‡ FORBES, FIRNLEY and MASON. *Ibid.*, 1933, **30**, 957.

sciousness results in a few seconds. Subsequently, as oxygen lack and carbon dioxide excess act on the vasoconstrictor centres, the arterial pressure rises, and if it then exceeds the intracranial pressure, the circulation through the brain recommences, aided by the considerable vasodilatation following on the period of anæmia ; if it does not rise high enough to overcome the intracranial pressure, death results owing to asphyxia of the medullary centres.

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CHAPTER XXXVI

EXAMPLES OF CIRCULATORY ADAPTATION

1. THE CIRCULATORY CHANGES DURING MUSCULAR EXERCISE

IN the preceding chapters we have analysed somewhat artificially the factors which are normally involved simultaneously in the adaptation of the circulation to the necessities of the body. In order to effect a synthesis of these isolated mechanisms, and trace out the chain of events concerned in the intimate co-operation of the circulation with other systems and organs of the body, we may take as an example the adaptations which are involved in muscular exercise. For the analysis of the different events in the circulation, we have hitherto had large recourse to animal experiments; but with the facts thus gained at our disposal, we can proceed to investigate the subject on man.

On initiating such experiments we meet at once with a new fact—viz. that under normal circumstances the reflex and automatic adaptation of the heart and vessels is forestalled and reinforced by the intervention of impulses proceeding from the brain. Thus, the willed effort, or the emotion of fear or anger which normally initiates extensive muscular movements, gives rise at the same time to impulses, starting in the brain, which excite changes in the circulatory and respiratory systems such as anticipate those which will be later excited reflexly or automatically as a result of the exercise. With a man seated on a stationary bicycle the mere question "Are you ready?" evokes increase of muscular tone in the act of attention, increased pulmonary ventilation, and a rise of pulse rate and of blood pressure. And these changes are increased as soon as the word "go" is given and the man starts to pedal, *i.e.* before the increased metabolic changes in the muscles can have had time to affect the medullary centres, or the muscular contractions the vigour of the circulation. The reinforcing impulses from the cortex, which stimulate the medullary centres and put these various mechanisms into action, are effective especially at the beginning of muscular work. In any steady work produced without particular effort or attention, the subsequent adaptations of the different organs of the body are probably chiefly automatic, the central reinforcing impulses being of especial importance when, under emotional stress of any description, the animal has to put forth its maximum effort.

We have seen that the oxygen intake, and the CO_2 output, may undergo at least a twelve-fold augmentation during violent muscular effort carried out for a short time; as much as a twenty-fold increase has been observed, and a five-fold increase is common, and may last for many hours. A necessary condition of all muscular exercise is that the muscles shall be supplied with oxygen in proportion to their requirements. Since the arterial blood is, under normal conditions at rest, about 95 per cent. saturated with oxygen, such increases in the oxygen usage by the muscles must imply a great increase in the blood supplied to them, though a certain extra amount of oxygen

may be gained by a more complete utilisation of the oxygen of the blood as it flows through the working tissues. This increased oxygen traffic involves, in its turn, an increase in the blood flow through the lungs and in the ventilation of the lungs. The circulation through the lungs has been investigated in the healthy man during rest and during exercise.*†‡ In Fig. 448 are shown results obtained by Means and Newburgh. It will be seen that the blood flow through the lungs, *i.e.* the output of the right ventricle per minute, and also the lung ventilation, increase in a manner almost absolutely proportional to the consumption of oxygen, and that all three increase *pari passu* with the work done per minute. How is this admirable adjustment of the activity of the heart and circulation to the oxygen needs of the muscles effected ? The output of the heart depends on the inflow into it, so that our

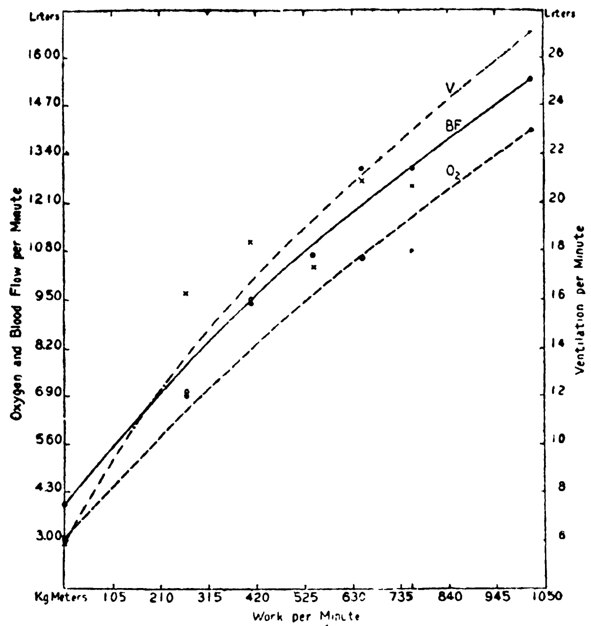

FIG. 448. Chart showing the Effect of Increasing Amounts of Muscular Work on the total Ventilation of the Lungs, V, on the Blood Flow, BF, and on the Oxygen Absorption, O_2. (From MEANS and NEWBURGH.)

problem is to determine the factors which increase this. At or before the onset of muscular exercise, unless this is quite moderate, there is relaxation of the muscle vessels and contraction of the splanchnic vessels, so that the increased output of blood is passed to the muscles, and later on also to the skin, rather than to the viscera; the latter, owing to the raised arterial pressure, may receive about the same amount of blood as in rest. Other things being equal, it is evident that, since the blood moves in a circle, any circumstance which favours the passage of blood through the circuit as a whole will lead to an acceleration of circulation rate, and this will take the form of a more rapid return of blood to the heart. But, as a matter of fact, there will also be accompanying changes operating in the opposite direction,

* KROGH and LINDHARD. *J. Physiol.*, 1913, **47**, 112.
† MEANS and NEWBURGH. *J. Pharmacol.*, 1915, **7**, 449.
‡ BOCK *et al.* *J. Physiol.*, 1928, **66**, 136.

by which the result is to some extent complicated. Thus, if we imagine a simple circulation, it is clear that extensive dilatation of the arterioles will give rise to the following chain of events :

Dilatation of arterioles → Increase of capacity and lowered resistance →
→ Fall of arterial pressure (which slows circulation rate).
↓
Cardiac acceleration
↑
→ Rise of venous pressure
↓
Increased output
(which accelerate circulation rate).

In consequence of such changes, a tendency to a fall of arterial pressure is more than compensated for by the accompanying alterations in the cardiac output. This is further augmented in exercise in a variety of ways. Probably the prime mover in accelerating the venous return, and so the circulation rate, is the effect of the contractions of the muscles themselves upon the rate of blood flow through their vessels. Opinion is divided as to whether during actual contraction the flow through the muscle is stopped * or is unaffected or even increased.† But during and after relaxation it is agreed that the flow is increased, sometimes greatly. In the larger veins, by reason of their valves, the muscular contractions tend always to drive the blood onwards to the heart. The more frequent and powerful the contractions of the muscles, therefore, the more rapidly the blood which enters them is passed on with force towards the big veins and the heart. The circulation through the big veins of the abdomen and chest is aided by the respiratory movements, which are also augmented in proportion to muscular activity, each inspiration driving the blood out of the big veins in the abdomen and aspirating it into the thorax. The blood flow into the heart is thus increased in proportion to the activity of the muscles. The first effect of muscular exercise will be to increase the heart output at each beat. The increasing tension on the venous side of the heart evokes reflexly a quickening of the heart rhythm, chiefly by inhibition of the vagus tone, possibly also by reflex stimulation of the sympathetic accelerator nerves. Further increase in the inflow into the heart is met by corresponding quickening of the heart rhythm and the output of each ventricle per minute is increased seven to ten times. The part played by increase of output per beat, and by increase of pulse rate respectively, in augmenting the total output

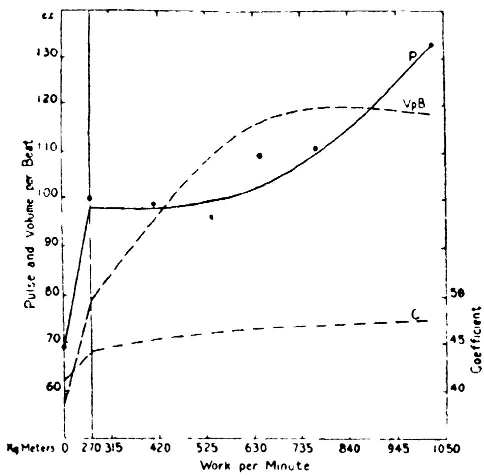

FIG. 449. Chart showing the Effect of Increasing Amounts of Muscular Work on the Pulse Rate, P, shown by dots ; on the Heart Output per Beat, VpB, and on the Co-efficient of Oxygen Utilisation in the Blood, C. (From MEANS and NEWBURGH.)

* ANREP *et al.* *Proc. R. S.*, 1934, **114B**, 223, 245 ; *J. Physiol.*, 1935, **85**, 375.
† REIN *et al.* *Ztschr. f. Biol.*, 1930, **90**, 260.

of the heart is shown in Fig. 449. In this figure, the first rise in pulse rate from sixty-eight to ninety-eight, and the corresponding increase in output per beat, can be regarded as associated with the initial changes originated by the act of attention and volition. It will be seen that between 270 and 600 kilogrammetres of work per minute the pulse rate remains practically unchanged, while the output per beat increases steadily with the work. After this point, there is very little further increase in the output per beat, which towards the end begins to diminish, while there is a steady increase in the pulse rate.

By this means, the blood is driven through the lungs at a rate corresponding to the increased needs of the muscles for oxygen. The passage of this blood through the muscles is provided for by two mechanisms. In the first place, owing to the increased cardiac output, the arterial pressure is raised,* and since the muscular vessels are relaxed and the splanchnic ones constricted, a large fraction of the circulating blood can be driven through the working tissues. The total available blood is increased by contraction of the spleen and by partial emptying of the portal system. In the second place, the muscles in their activity produce various metabolites, which cause further dilatation of the arterioles and capillaries in the muscles themselves. A third way in which muscular circulation is increased may be by reflexes initiated in the tissue by the pressure of metabolites formed by contraction. During rest, it is probable that the majority of the capillaries are closed; during activity these dilate and are filled with blood, so that the capillary bed in the muscles may be increased many times in area, and each element of the muscle is brought into close relation with a dilated capillary, through which is flowing a rapid stream of oxygenated blood. Krogh † has shown that the number of blood-containing capillaries in each square millimetre cross-section of the muscle may be increased 40 to 100 times during maximal activity of the muscle. As a result, the oxygen tension in the muscle fibres becomes almost equal to that in the capillaries themselves.

Complex physico-chemical changes taking place in the blood ‡ aid the dissociation of the oxyhæmoglobin passing through the capillaries, and therefore set free oxygen for the use of the muscles. On this account, we find almost invariably that the utilisation of the oxygen taken in from the lungs is more complete during exercise. Except during the most exhausting exercise the arterial blood is as fully saturated with oxygen as when at rest,§ but in working to exhaustion it may fall to 85 per cent. saturation.‖ The fraction of the oxygen content of the arterial blood which is abstracted as it flows round the circulation is known as the 'co-efficient of utilisation.' Thus if 328 c.c. of oxygen were used per minute and the blood flow were 4·5 litres per minute, $\frac{328}{4 \cdot 5} = 73$ c.c. oxygen would be utilised per litre of blood. If the oxygen capcity of the blood were 193 c.c. per litre, the co-efficient of utilisation would be $\frac{73}{193} = 0 \cdot 38$. In Fig. 449 the co-efficient of the oxygen utilisation is given by the curve C. It has been found that the co-efficient of utilisation rises during severe exercise from a resting value of about 0·3—0·5 to 0·7—0·8.¶

* L. Hill. *J. Physiol.*, 1898, **22**, *Proc.* xxvi.; Bowen. *Amer. J. Physiol.*, 1904, **11**, 59.
† Krogh. *J. Physiol.*, 1919, **52**, 409, 457.
‡ Hochrein, Dill and L. J. Henderson. *Arch. f. exp. Path. u. Pharm.*, 1929, **143**, 129.
§ Himwich and Barr. *J. Biol. Chem.*, 1923, **57**, 363.
‖ Harrop. *J. Exp. Med.*, 1919, **30**, 241.
¶ Douglas and Haldane. *J. Physiol.*, 1922, **56**, 69.

It has been shown by Cannon that every state of excitement, and especially fear and anger, is attended with increased secretion of adrenaline into the blood stream. During violent exercise associated with emotional stress, there will be an excess of adrenaline circulating in the blood, and this will reinforce the activity of the circulation. Thus, it will increase the constriction of the splanchnic area, already excited reflexly and by the central effects of the increased CO_2 or lactic acid in the blood. In the heart, the adrenaline will increase the contractile power and also the rate of beat, while, by its dilator action on the coronary vessels, it will aid the supply of oxygen to the heart muscle. At the same time, it will cause a rapid conversion of the glycogen of the liver into sugar, so that the contracting muscles will be adequately supplied with the food which they can utilise with the greatest ease. It is doubtful whether these adjuvant effects of adrenaline are to be reckoned with, except in cases of severe emotional stress.

After the conclusion of exercise, the changes which accompanied it disappear. Arterial pressure falls very quickly, and usually drops to below the normal, slowly to return. Pulse rate also drops very suddenly in those with efficient hearts, and sometimes shows a second slight rise accompanying the rapid fall of blood pressure. Oxygen usage and cardiac output show a more gradual decline, especially after severe exercise.

Training. The muscular efficiency of a man is measured by the extent to which he can call upon his body for increased efforts, *i.e.* by his margin of response. Over a moderate period of time, the individual may increase his muscular work, his respiratory exchange, and the rate of his circulation six times above that which obtains during rest. In a sudden or short-lasting effort, a much greater amount of energy can be put out, the muscles 'going into debt' for the necessary oxygen. The performance of a normal individual can be increased by training, the essential features in which are graduated exercise and healthy diet, so that the muscle grows and becomes free from interstitial fat, while the fluid parts of the body and of the blood are diminished so that a larger amount of oxygen can be carried per unit volume of blood. The well-trained individual may have a margin of as much as 1200 per cent. Disease is marked by a diminution of the margin. In Fig. 450 is given diagrammatically the response of the circulation and respiration of a man with heart disease affecting the aortic and mitral valves. This man had no discomfort and was able to do ordinary work without ill effects. On testing him on measured muscular tasks, it will be seen that, although at first he reacts

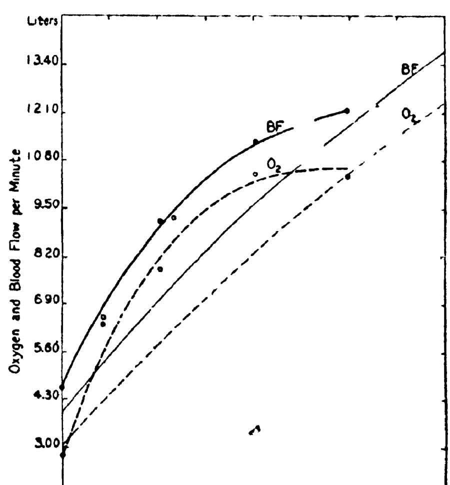

FIG. 450 Chart showing the Effects of Muscular Work on the Blood Flow and Oxygen Consumption in a subject with Aortic Disease, as compared with a normal individual (shown in lighter lines).
(From MEANS and NEWBURGH.*)
(For oxygen consumption omit the decimal point and read in c.c.s.)

* MEANS and NEWBURGH. *J. Pharmacol.*, 1915, 7, 441.

like the normal individual, his margin is diminished, and when doing only 315 kilogrammetres of work per minute, the rise in the oxygen intake and in the heart output fails to keep pace with the increase in the work and also loses the parallelism which is so marked a feature in normal individuals. With increasing disease, the time would finally come when the margin was reduced to 100 per cent. or 50 per cent., so that even the act of changing from a recumbent to an erect position might be too much for the enfeebled adaptive mechanisms of the body, and the patient would have to keep to his bed. There is thus no definite dividing line between health and disease, the change from one to the other being but a progressive diminution of margin or extent of adaptation.

We have seen that the physiological condition of the heart is measured by the dilatation of its cavities, *i.e.* of the shortest length of its muscle fibres, required in order that in its beat it may set up a contractile stress adequate to expel its contents against the arterial resistance. Thus a degree of filling of the heart, which in a well-trained man may be adequate to ensure a contraction sufficient entirely to empty its cavities, in a weaker heart would be inadequate, so that blood would accumulate at each diastole until the stretching of the fibres was sufficient to ensure that the amount entering during diastole was expelled at each systole. The trained man—*i.e.* with a heart in good condition—will therefore have a considerable range over which the output per beat can be increased with increasing inflow, without alteration of rhythm. In the untrained man this margin will be smaller, so that the second mechanism of adaptation, viz. quickening of the heart beat, will be sooner brought into action to cope with the increased inflow associated with muscular exercise. Thus, one finds a considerable difference in the effect of exercise on the pulse rate in trained and untrained individuals respectively,† and this is especially shown in the promptness of recovery of the pulse when the exercise comes to an end, the effects lasting much longer in the untrained. In all cases exercise not carried to exhaustion tends to be followed by a prolonged diminution both in pulse rate and in blood pressure (*cf.* Fig. 451).

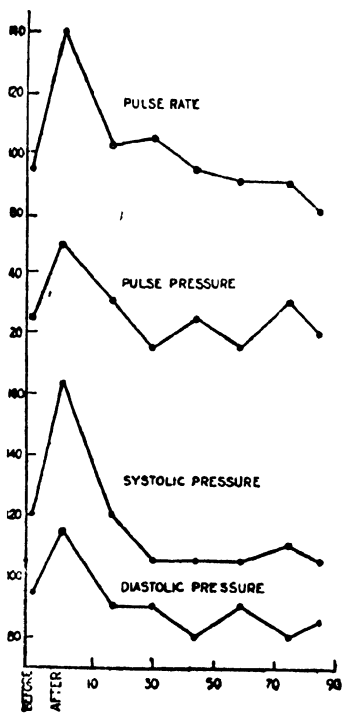

FIG. 451. Curves showing the Influence of Exercise on the Circulation. The exercise was a six-mile run. Ordinates = mm. Hg pressure and rate per minute. (O. S. LOWSLEY.*)

Ischæmic Pain. When muscles contract under such conditions that the blood supply does not increase in keeping with their increased needs, a peculiar cramp-like pain is experienced, in the muscles concerned. It is met with in cases of narrowing of the arteries to a limb, and the condition is called intermittent claudication. It may be

* LOWSLEY. *Amer. J. Physiol.*, 1911, **27**, 446.
† PEMBREY and TODD. *J. Physiol.*, 1908, **37**, *Pr.* lxvi.

imitated by placing a sphygmomanometer armlet round the upper arm, inflating it suddenly to 250 mm. Hg, and then making gripping movements of the hand every second. The pain commences in about 35 seconds and becomes intolerable in 70 seconds; it persists as long as the occlusion of the circulation is kept up, but, on restoring it, is abolished in 3 seconds. If the circulation is again stopped, no pain is felt unless the muscles are once more exercised, but, if they are, it appears much sooner than in the first instance. These various facts led Lewis * to the conclusion that the pain is due to the accumulation during muscular contraction of a substance, which he called the "P-factor"; this under normal conditions is removed by oxidation, but when this is not done the substance stimulates pain fibres. The cardiac pain accompanying coronary occlusion is believed by Lewis to be explicable in a similar way.

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2. THE INFLUENCE OF VARIATIONS IN THE TOTAL QUANTITY OF BLOOD

PLETHORA AND HYDRÆMIC PLETHORA. The effects of increasing the total volume of circulating fluid may be studied by injecting several hundred cubic centimetres of defibrinated blood or normal saline solution into a vein. The condition resulting has been called *plethora* or *hydræmic plethora* respectively. They differ inasmuch as in the latter state the plasma is made more dilute and the corpuscular concentration and blood viscosity lowered. On the arterial pressure the result of such an injection is not very marked. There is a slight initial increase in the pressure, but the increase is by no means proportional to the amount of fluid injected, showing that the excess fluid is not to any large extent contained in the arterial system. On examining the pressure in the veins, however, we find a very great relative rise of pressure, and on opening the abdomen it is seen that all the veins are distended and that the liver is swollen. This is in part due to the effect of lowered viscosity of the blood, which now passes more readily through the finest vessels. The organism further prevents the rise on the arterial side by relaxing the whole system of arterioles, so that the distribution of pressures is altered, and the venous approximates more closely to the arterial pressure. This arterial dilatation and lessened viscosity augments the velocity of the blood: *e.g.* the velocity may be accelerated to six or eight times the normal rate by injecting an amount of salt solution equivalent to 50 per cent. of the total blood.

The high venous pressure causes increased diastolic filling of the ventricles, and therefore augments the strength of the beat. If the vagi are intact, the frequency is also reflexly raised, in consequence of the greater distension of the auricles. Thus, the work of the heart is increased in three ways—viz. by

(1) Rise of arterial pressure.
(2) Greater frequency of beat.
(3) Increased output at each beat.

* LEWIS. *Arch. Int. Med.*, 1932, **49**, 713.

These series of changes result in the relief of the vascular system. The rise of pressure in the aorta causes a reflex dilatation of all the arterioles of the body, through the afferent fibres of the cardio-aortic and carotid sinus nerves. The heightened pressure in the abdominal veins and capillaries, together with the lowered colloid content of the blood, if saline is given, causes a great leakage of fluid in the form of lymph, from the capillaries of the

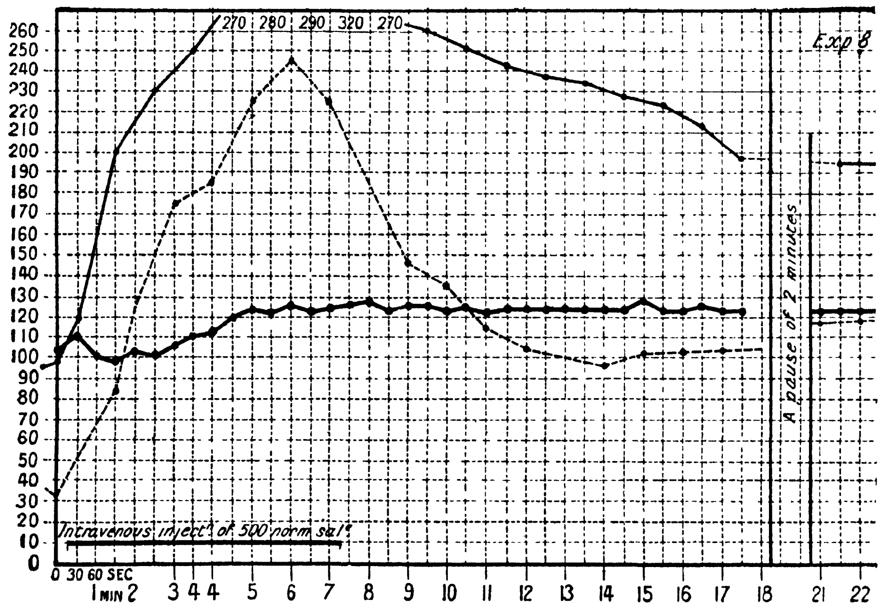

FIG. 452. Effects of Hydræmic Plethora on the Pressures in the Carotid Artery (thick line), Portal Vein (thin line), and Inferior Vena Cava (dotted line). (BAYLISS and STARLING *.)

The arterial pressure is in mm. Hg ; the venous pressures in mm. H_2O.

intestines and liver, while similar changes in the blood in the glomeruli of the kidney induce a copious secretion of urine, so that within a couple of hours after the injection of salt solution, the volume of the circulating fluid may have returned to normal.

This recovery is effected with greater difficulty if the plethora has been brought about by the injection of blood, or of saline containing 8 per cent. of gum arabic, since these fluids exert a colloidal osmotic pressure, and cannot escape so rapidly from the capillaries, nor be excreted unchanged by the kidneys. Hence it is easy to kill an animal by wearing out its heart, if too large quantities of blood be injected.

HÆMORRHAGE AND ANÆMIA. Any diminution of the total volume of the blood, as by bleeding, would tend to lower the pressure on both sides of the system. The vasomotor centre, however, reacts to maintain the normal arterial pressure, and so the circulation through the brain, unaltered. This is attained reflexly by an excitation of the sympathetic system, which includes an outpouring of adrenaline into the blood stream. In consequence there is considerable vascular constriction, which diminishes the total capacity of the system and keeps the blood as much as possible on the arterial side.

* BAYLISS and STARLING. *J. Physiol.*, 1894, **16**, 159.

Hence, a slight loss of blood has no influence on the arterial blood pressure, and even considerable loss may only cause a small reduction, but it causes a fall of pressure in the veins, increased respiratory movements, blanching of the skin, an increase of blood sugar, and diminished flow of urine. The heart beats more frequently, and so aids in emptying the venous into the arterial system.

The compensatory vasoconstriction produced by hæmorrhage is, however, not exerted equally or indiscriminately throughout the body.* It is greatest in the skin, and next in order come the spleen, muscle, glands, and mucous membranes: the alimentary canal and organs concerned with swallowing are less affected. This is a reasonable adaptation, since if absorption were affected, as it would be if there was widespread splanchnic constriction, the lost blood could not be readily replaced.

The constriction of skin vessels after hæmorrhage persists for an hour or more after the removed blood has been replaced by transfusion. Even if more blood is restored than was lost the constriction still remains for some time, though a generalised reactive hyperæmia might have been expected.† Thus the capacity of the circulatory system is kept small and shock is warded off.

The deficiency of circulating fluid caused by bleeding is soon remedied by a transfer of fluid from the tissues to the blood. This transfer is independent of the flow of lymph from the thoracic duct into the blood, and is the direct consequence of the universal fall of capillary pressure which results from the bleeding. The abstraction of fluid from the tissues is responsible for the extreme thirst which is one result of hæmorrhage. The transfer of fluid from tissues to blood is extremely rapid; even during the course of a bleeding, it is found that the later samples of blood are more dilute than those obtained at the beginning. The blood also clots more rapidly. Thus the loss of fluid is replaced and further bleeding checked. After a bleeding, however, an animal has lost proteins and blood corpuscles, and these constituents of the blood are but slowly restored, the former indirectly from the food, the latter by an increased activity of the red marrow.

PHYSIOLOGICAL ALTERATIONS IN THE FILLING OF THE VASCULAR SYSTEM. The volume of blood in circulation is not quite constant, but often undergoes small and rather rapid fluctuations owing to an altered balance of loss and gain of fluid through the capillary walls. Thus, a rise of general arterial pressure, or of capillary pressure, is accompanied by concentration, and a fall by dilution, of the red corpuscles, as if to compensate. This alteration of corpuscular content is, however, in part due also to alterations in the volume of the spleen, as is explained later.

The general capacity of the vascular system is also variable. Great variations can be produced by the opening or closing of the capillary network in large masses of tissue. Among the larger vessels the most variable in capacity are the great veins; these in the human subject can hold 500 c.c. or more when distended, but usually they form baggy reservoirs of very variable content. The capacity of the venous system is very important in relation to the portal circulation, discussed in an earlier chapter. The portal vein, with its rootlets, represents a reservoir which when distended can hold about a third of the blood in the body. Partial depletion of the portal venous system is what happens, for instance, in exercise; the result is a fall of portal pressure, a rise of caval pressure, and a greatly increased

* ROUS and GILDING. *J. Exp. Med.*, 1929, **50**, 189 GILDING. *J. Exp. Med.*, 1929, **50**, 213.

† GILDING and ROUS. *J. Exp. Med.*, 1929, **50**, 471

venous return and cardiac output, which is due to a virtual increase in the amount of blood in the circulatory current.

The Spleen. The spleen may be regarded as a further special part of the portal system which, among other functions, acts especially as a reservoir for red cells. The investigations of Barcroft showed that the size of the spleen varies with physiological requirements, as such a view would anticipate. It may be extirpated without ill effect, and occasionally is congenitally absent.

This organ is formed of a framework of connective tissue and unstriated muscular fibres, in the interstices of which is contained the *splenic pulp*. This consists of a fine fibrillar network the meshes of which contain the cells of the splenic pulp. These are monocytes, and closely related cells giving origin to them, red cells and leucocytes. The blood from the splenic pulp is collected into large venous sinuses which run along the trabeculæ to the hilum, where they unite to form the splenic vein. These sinuses are lined by cells belonging to the reticulo-endothelial system, and called histiocytes or macrophages. They, and the monocytes of the pulp, are actively phagocytic.

The arteries to the spleen are beset in their course along the trabeculæ with small nodules of lymphoid tissue, which are known as the Malpighian follicles. These contain germinal areas in which, by division, new lymphocytes are produced, and find their way into the blood. The arteries yield a few capillaries which supply the Malpighian follicles, but for the most part open either directly into the venous sinuses, or else, by way of curious perforated sacs, into the pulp. From the pulp, the blood can pass by numerous spaces into the venous sinuses, and so into the splenic vein.

There are thus alternate paths for the circulation through the spleen; that from arteries direct to sinuses is probably free, but it is evident that

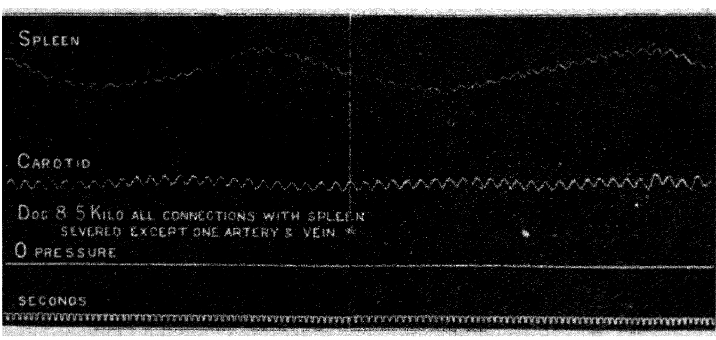

FIG. 453. Plethysmographic Tracing of Spleen (upper curve) from a Dog, showing the Spontaneous Contractions of this Organ. (Reduced from a tracing by SCHAFER.)

the blood must meet with considerable resistance in passing through the close meshwork of the splenic pulp. This path of the circulation is, however, assisted by the occurrence of rhythmic contractions of the plain muscle forming the capsule and trabeculæ. If the spleen volume be recorded, it will be seen to be subject to a series of slow variations, each contraction and expansion lasting about a minute and recurring with great regularity (Fig. 453). Superposed on these larger waves are smaller passive undulations which correspond to the respiratory and cardiac fluctuations of arterial blood pressure. In addition to these rhythmic

variations in size the spleen can also, by its plain muscle, alter its mean volume very greatly.

In spite of these rhythmic contractions, however, the normal pulp circulation is probably very sluggish. Thus when rats were slowly poisoned with carbon monoxide, it was a long time before the splenic blood, drawn by puncture, became as saturated with CO as that in the general circulation.* The alteration of the mean volume of the spleen is under the control of the nervous system, and a rapid and extensive contraction may be induced by stimulation of the splanchnic nerves,† and during asphyxia or anoxæmia.‡ Under these conditions, a considerable volume of blood, which is very rich in red cells, is expressed into the general circulation. In the cat, it was estimated that one-sixth of the total blood volume, or one-third of the total red cells, could be so expelled from the spleen.

FUNCTIONS OF THE SPLEEN. A concensus of opinion considers the spleen to have important functions in connection with the destruction of red blood cells. Erythrocytes of the splenic pulp are less resistant to hypotonic salt hæmolysis and more resistant to saponin hæmolysis than are the red cells of the general circulation.§ After removal of the spleen the hypotonic fragility of the red cells is for a time reduced. Microscopic examination shows that the macrophages and monocytes of the spleen are full of particles of brown iron-free pigment (hæmofuscin) or fragments of red corpuscles,|| and we have already discussed the destruction of red corpuscles and the formation of bilirubin in these cells (p. 539). Part of the iron liberated from the disintegrated hæmoglobin is retained in the reticulo-endothelial cells in some form of combination with other substances (hæmosiderin). Probably most of the iron-containing substances are carried away by monocytes and detached reticulo-endothelial cells and temporarily stored in the liver. In some forms of anæmia (associated with hæmolytic jaundice) the reticulo-endothelial cells in the spleen are over-abundant and hyperactive and the spleen is enlarged; relief of the condition is effected by removal of the spleen, after which operation the number of red cells in the blood is increased and the bilirubinæmia is reduced.

In many cases of infectious disease, such as recurrent fever, the splenic phagocytic cells are observed towards the end of the attack to be full of the organism—a spirillum—which is the cause of the disease. In fact, these cells can take up foreign solid particles of all sorts held in suspension in the blood plasma. We must, indeed, look upon the spleen as the great blood filter, purifying the blood in its passage by taking up particles of foreign matter and effete red corpuscles. It also produces lymphocytes and monocytes, as we have already seen. In embryonic life, and in the adult in certain types of anæmia, it also produces leucocytes and red cells, just as the red marrow does.

Barcroft has shown that the spleen may serve as a reservoir of red blood corpuscles. When there is a deficiency of hæmoglobin or of oxygen in the blood, the spleen contracts, driving some of the corpuscles it contains into the general circulation.¶ Thus it contracts in exercise, in asphyxia, after hæmorrhage, &c. to such an extent that it may be reduced to a fraction

* BARCROFT, J., and BARCROFT, H. *J. Physiol.*, 1923, **58**, 138.

† ROY. *J. Physiol.*, 1881, **3**, 203; BARCROFT *et al.* *J. Physiol.*, 1932, **74**, 321.

‡ SCHAFER and MOORE. *J. Physiol.*, 1896, **20**, 1.

§ ORAHOVATS. *J. Physiol*, 1926, **61**, 436.

|| ADDISON. *Amer. J. Anat.*, 1919, **26**, 437; CUNNINGHAM, SABIN and DOAN. *Proc. Soc. Exp. Bio.*, 1923, **21**, 326.

¶ BARCROFT *et al.* *J. Physiol.*, 1925, **60**, 443; BARCROFT and STEPHENS. *J. Physiol.*, 1927, **64**, 1.

of its former size (Fig. 454). No doubt the liberation of adrenaline into the blood also greatly assists the contraction of the spleen under these conditions.

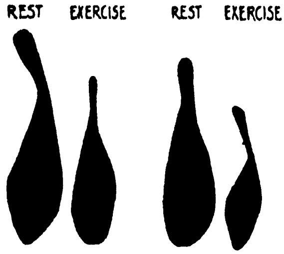

FIG. 454. Shrinkage of the Spleen of the Cat with Exercise. Reconstruction from X-ray outlines as seen from the side. (BARCROFT, *Lancet*, 1925 (I.), **208**, 319.)

After removal of the spleen, carbon monoxide is more rapidly fatal than to normal animals.*

Dale and Dudley have shown that the spleen of some animals is particularly rich in esters of choline. Whether the presence of this potent substance has any special significance is at present unknown.

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* BARCROFT *et al.* *J. Physiol.*, 1925, **60** 79.

CHAPTER XXXVII

LYMPH, TISSUE FLUIDS, AND CEREBRO-SPINAL FLUID

In all parts of the body the blood flows in capillaries with definite walls, and is thus separated from the tissue-elements by these walls and by a varying thickness of tissue. In some organs, such as the liver and lung, every cell is in contact with the outer surface of some capillary; while in others such as cartilage (which is avascular), a considerable thickness of tissue may separate any given cell from the nearest capillary. A middleman is thus needed between the blood and the tissues, and this middleman is the *tissue fluid*, which fills spaces between all the tissue elements, so that any tissue can be regarded as a sponge soaked with fluid exuded from the blood capillaries.

Throughout these spaces we find a close network of vessels, lined and separated from the tissue spaces * by a layer of extremely thin endothelial cells, and this plexus communicates with definite channels—lymphatics, by which any excess of fluid (*lymph*) is drained off. New lymphatic vessels have been seen to grow by sprouting from previously existing lymphatics.† The lymphatics all run towards the chest, where those of the hind limbs join a large vessel (the *receptaculum chyli*), which receives the lymph from the alimentary canal, to form the thoracic duct. This, after receiving the lymphatic trunks from the left fore limb and the left side of the neck, opens into the venoussystem at the junction of the left internal jugular with the subclavian vein. A small vessel on the right side drains the lymph from the right fore limb and right side of the chest and neck.

The lymph may be looked upon as derived from the tissue fluid which exudes from the plasma, through the capillary wall, bathes all the tissue elements, and contributes to the formation of secretions of glands; the excess of fluid (Fig. 455) passes between or through the endothelial cells into the peripheral lymphatic network, whence it is carried by lymphatic trunks into the thoracic duct, by which it is returned again to the blood.

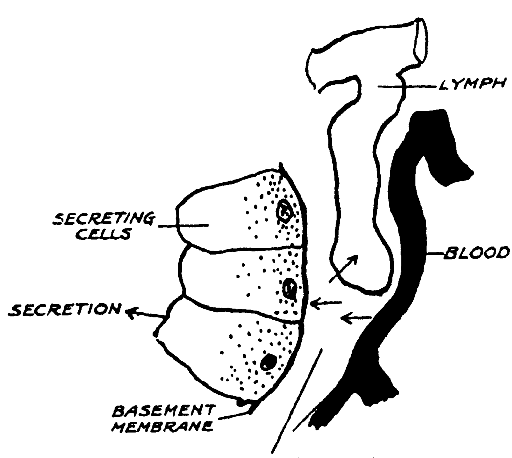

Fig. 455.

It is easy to obtain lymph for examination by putting a cannula into the thoracic duct, and collecting the fluid that drops from it. We may also

* MacCallum. *Arch. f. Anat. u. Phys.* (*Anat. Abt.*), 1902, 273.
† Clark and Clark. *Amer. J. Anat.*, 1938, 62, 59.

tap in a similar way one of the large lymphatic trunks of the limbs*; but in the latter case we often have to use artificial means to induce a flow of lymph, since little can be obtained from a limb at rest, the only part of the body whence there is normally a constant flow of lymph being the alimentary tract.

The tissue fluid is thus truly a middleman; as any substance, oxygen or foodstuff, is taken up by a tissue cell from the fluid surrounding it, this latter recoups itself at once at the expense of the blood. Thus there would seem to be no need for lymphatics to drain a limb, were it not that under many conditions, which we shall study directly, the exudation of fluid from the blood vessels is so excessive that, if it were not carried off at once and restored to the blood, it would accumulate in the tissue spaces, give rise to œdema, and by pressure on the cells and blood vessels affect them injuriously.

PROPERTIES OF LYMPH. Lymph obtained from the thoracic duct varies in composition and appearance. From a fasting animal the lymph is a transparent, slightly yellowish liquid. When obtained from an animal shortly after a fatty meal, it is milky from the presence of minute particles of fat absorbed from the alimentary canal, and, if the intestines be exposed, the small lymphatics are to be seen as white lines running from the intestine to the attached part of the mesentery. It is owing to this fact that these lymphatics have received the special name *lacteals*, the lymph in them, when milky, being called *chyle*.

On microscopic examination, the lymph presents lymphocytes similar to those of blood, which are partly derived from the lymph. All the lymphatics pass at some point of their course through lymphatic glands, which add lymphocytes. Lymphocytes are also formed in all localities where we find adenoid tissue, such as the tonsils, air passages, Peyer's patches and solitary follicles, Malpighian bodies of the spleen, and thymus.

The lymph from the thoracic duct clots slowly after it has left the vessels, forming a colourless clot of fibrin. It contains about 6 per cent. of solids which are similar to those of the plasma, and are present in similar proportions except that it contains much less protein, as the following table shows:—

COMPARISON OF SERUM AND LYMPH OF DOG. (ARNOLD and MENDEL, *J. Biol. Chem.*, 1927, **72**, 189.)

	Serum g/100 c.c.	Lymph g/100 c.c.
Total solids	8·3	5·2
Chlorides	0·392	0·413
Calcium	0·0104	0·0093
Phosphorus	0·0043	0·0036
Glucose	0·123	0·124
Non-protein N.	0·0272	0·0270
Protein N.	0·90	0·57

Anything causing a change in composition of the blood, *e.g.* feeding with glucose, injection of insulin or parathyroid hormone, removal of the kidneys or parathyroids, &c. causes a comparable change in the composition of the

* WHITE, FIELD and DRINKER. *Amer. J. Physiol.*, 1933, **103**, 34.

lymph.* Liver lymph may contain more sugar than that from other parts if hepatic vein blood contains more sugar than portal vein blood.†

THE PRODUCTION OF LYMPH. The total volume of lymph in the body is no doubt variable from time to time, but is probably of about the same order as the blood volume. The volume of tissue fluid is probably very small under normal conditions, though much increased when tissues become œdematous. Investigation has shown that in the formation of tissue fluid and lymph the endothelial cells act passively, as colloidal membranes of variable permeability. The factors which are responsible for the transudation of tissue fluid may be divided into two classes—mechanical and chemical, the former depending largely on the pressure of the blood in, and the degree of active dilatation of, the vessels, and the latter chiefly on the metabolism of the cells outside the vessels.

If the formation of tissue fluid may be compared to a process of filtration, the amount of it formed in any given capillary area must be dependent on the capillary permeability, and on the difference of pressure between the blood in the vessels and the fluid in the tissue spaces. This latter pressure is normally extremely low (about 20 mm. H_2O),‡ so that we should expect that the exudation will rise and fall as the capillary pressure is increased or diminished. The chief factor is the permeability of the walls of the blood (and lymph) capillaries.§ Whereas the capillary walls in the limbs and connective tissues present a considerable resistance to the filtration of lymph, and keep back the larger portion of the proteins of the blood plasma, the intestinal capillaries are much more permeable, while in the liver we find the greatest permeability. As regards the interchanges of fluid and non-colloidal solutes between blood stream and tissue fluid, it seems clear that there is a sort of circulation of fluid constantly going on. The blood pressure in a capillary falls off sharply from its arterial to its venous end. Often it may be 30 to 40 mm. Hg. at the former and only 10 to 15 at the latter. At the higher pressure, which is above that of the colloidal osmotic pressure of the plasma, fluid will be passed out by ultrafiltration; at the further end the pressure is below that of the colloid osmotic pressure of the plasma, and so at the venous end fluid will be reabsorbed into the blood stream.

If we regard the lymph as being completely separated from the tissue fluid by the delicate walls of the lymphatic capillaries, the lymph flow will similarly depend on the relation between pressure of tissue fluid and permeability of lymphatic capillary. It has been the usual practice to consider the permeability of blood and of lymph capillaries together. The justifiability of this may, however, be doubted, since there is reason to believe that the tissue fluid, even under normal conditions, and certainly under pathological ones, can vary considerably without there being any change in the rate of lymph formation.

The trivial lymph flow from a limb lymphatic is practically unaltered by changes in the arterial supply, although an increase may be obtained by active movement or by massage, or by ligaturing all the veins of the limb so as to cause a great rise of capillary pressure. It has been shown that when a person stands still in the erect posture, there is a loss of plasma, an increase in the red cell count, and a slow swelling of the legs. This indicates that fluid has passed out from the blood into the tissue spaces of the legs, the actual loss of blood volume being of the order of 300 or 400 c.c. in half an hour or less. On resuming the recumbent posture the process is reversed.

* Heim, Thomson and Bartter. *Amer. J. Physiol.*, 1935, **113**, 548.
† Clark and Winter. *J. Physiol.*, 1935, **83**, 49P.
‡ Wells *et al.* *J. Clin. Inv.*, 1938, **17**, 489.
§ Starling. *J. Physiol.*, 1894, **16**, 224.

The lymph flow from the intestines can be measured by collecting the lymph from the thoracic duct. If the lymphatics which leave the liver in the portal fissure be previously ligatured, almost the whole of the thoracic duct lymph in an animal at rest is derived from the intestines. It will be found that lowering of the capillary pressure in these organs, by obstructing the thoracic aorta, stops the flow of lymph absolutely, whereas a rise of capillary pressure, such as that produced by ligature of the portal vein, causes a four or five fold increase of the lymph. The lymph from the intestines is relatively rich in proteins, *i.e.* the endothelial membranes are here unusually permeable.

The effect of rise of capillary pressure on the lymph flow is still more striking in the case of the liver. If the inferior vena cava be obstructed just above the opening of the hepatic veins, there is a rise of pressure in the liver capillaries to three or four times the normal height, and a large increase in the lymph flow from the thoracic duct. The lymph may be increased eight to ten times in amount, and it contains more protein than before, in fact almost as much as the plasma. If the portal lymphatics be previously ligatured, obstruction of the inferior vena cava has no effect on the lymph flow, showing that the whole of this increase is derived from the one region of the body where the capillary pressure is increased, viz. the liver.*

It is possible to prepare artificial membranes, *e.g.* of collodion, which show similar gradations of permeability; some so slightly permeable that no colloids can be forced through them (*cf.* ultra filtration, p. 87), others allowing some colloids (of small size of particles) to pass, while retaining others, and some even allowing nearly all the colloids of the plasma to pass. When such a collodion membrane is used in an osmometer, with blood plasma inside and water or salt solution outside, the salts and other materials to which it is permeable will pass through, and the only osmotic pressure exerted will be that of the colloids which cannot permeate. Hence the higher the permeability of the membrane, the lower the effective osmotic pressure of the colloids, and the greater the amount of plasma constituents that will pass through. If the pressure inside the osmometer is raised above the osmotic pressure of the retained colloid, fluid and dissolved substances will pass out at a rate proportional to the raised pressure.

In the case of the limbs and connective tissues generally, the second factor, that of permeability, comes more into prominence. The osmotic interchanges between blood and cell, through the intermediation of the tissue fluid, are constantly going on in the normal life of the tissue, and are quite independent of the amount of fluid produced. Thus a gland cell may use up oxygen, calcium, or sugar, and create a deficit of these substances in the layer of tissue fluid immediately surrounding the cell. There is at once a disturbance of the equilibrium, and a flow of these substances from blood to tissue fluid is set up. Such changes can occur with great rapidity. We find, for instance, that if a very large amount (40 grammes) of glucose be injected into the circulation, osmotic equilibrium between blood and tissue fluid is established within half a minute of the termination of the injection. In this case, the rise of osmotic pressure caused by the injection of the sugar at once attracts water from the tissue fluid, and this in its turn from the tissue cells, while at the same time glucose is diffusing outwards from the blood, until finally the osmotic pressure inside and outside the vessels is the same. A process of this character would more usually work under normal circumstances in the reverse direction, and lead to a passage of fluid from blood to tissues and tissue spaces, and changes of this nature have often

* STARLING. *J. Physiol.*, 1895, **19**, 15.

been believed to be associated with activity of the tissue cells. Every active contraction of a muscle, for instance, is attended by the breaking down of a few large molecules into a number of smaller ones, and this causes a rise of osmotic pressure in the muscle fibre and surrounding fluid, and therefore a passage of fluid from blood to tissue. It is certain, however, that much of the increased lymph flow from a limb during contraction of its muscles is due to the mechanical effect of the movement in accelerating lymph flow, so that the factors involved are complex.

It has been supposed, by similar arguments, that when a gland, such as the submaxillary gland, is made to secrete, the secreting cells which have passed out fluid recoup themselves from the surrounding tissue fluid, raising the concentration of this fluid, and the difference in concentration thus caused, at once induces a passage of water from blood to tissues, and so perhaps to lymph. The evidence of experiment is, however, conflicting, for while several early workers claimed to have obtained an increase of lymph flow from the submaxillary gland when caused to secrete by chorda stimulation, others, more recently * have found no change in the lymph flow. This might be due to the fact that, although an increased amount of tissue fluid is exuded from the capillaries, the excess is all passed on into the secretion; against this explanation, however, is the further fact that if we paralyse the gland cells by means of atropine, the active flushing of the gland, which accompanies activity, produces little, if any, increase in the lymph flow from the gland.

When we speak of the permeability of the blood "capillaries" we should strictly include the smallest arterioles and venules with them. In muscle, where observations are easily made, the walls of the arterioles have been shown † to be slightly permeable, and those of the venules easily permeable, to molecules of dyestuffs which are probably larger than glucose molecules. In the capillaries proper, there is evidence that the permeability increases in passing from arteriole to venule. When we remember that the muscle capillary is a very long one, the advantage of this graded permeability is evident, for if the vessel were equally permeable throughout its length, the tissue near the venous end of it would have less opportunity to receive nutrient material or get rid of its waste products, because all the advantage of first choice would lie with the tissue at the arterial end.

It is probable that the permeability of the endothelium is increased when there is active dilatation of the capillaries, as happens when an organ becomes active. Further, the relative insusceptibility of the limb capillaries to pressure holds only for the absolutely normal capillary. Any factor which leads to impaired nutrition of the vascular wall, such as deficiency of supply of blood or oxygen, the presence of poisons in the blood or in the surrounding tissues, scalding or freezing, increases at the same time its permeability. Under such conditions, the limb capillary reacts to changes of pressure like a liver capillary, the slightest increase of pressure causing an appreciable increase in the production of tissue fluid. This increased production may be too great to be carried off by the lymphatic channels, so that the exuded fluid stays in the tissue spaces, distending them, and causing the condition known as *œdema* or dropsy.

Lymphagogues.‡ Among the substances which have a direct action on the vessel wall are a number of bodies which were described by Heidenhain as lymphagogues of the first class. As their name implies, these

* LEIGH. *Amer. J. Physiol.*, 1935, **112**, 657.
† ROUS, GILDING and SMITH. *J. Exp. Med.*, 1930, **51**, 807.
‡ STARLING. *J. Physiol.*, 1894, **17**, 30.

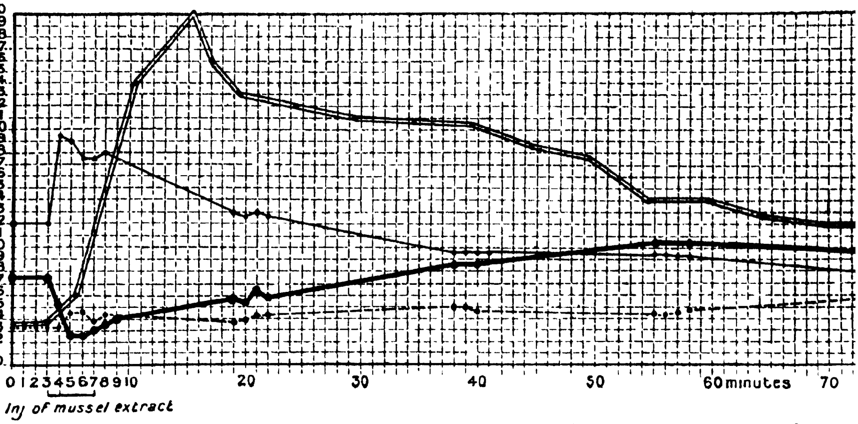

FIG. 456. Changes in Lymph Flow, and in Portal, Inferior Cava, and Arterial Pressures, resulting from Injection of a Member of the first class of Lymphagogues (Extract of Mussels). The double line = lymph flow in c.c. per ten minutes; thin line = portal vein; thick line = carotid artery; dotted line = inferior vena cava. (STARLING.)

A

B

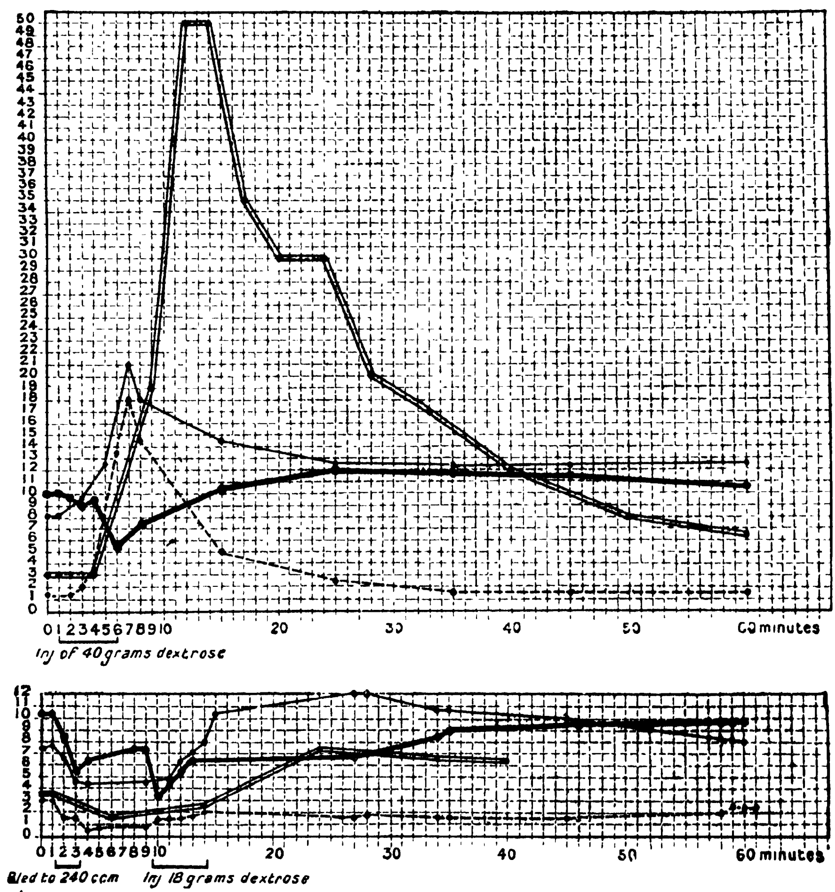

FIG. 457. Effect on Lymph Flow and on Arterial and Venous Pressures of Injection of Concentrated Solution of Glucose.
In B 240 c.c. blood was drawn from the animal before the injection. (STARLING.)
Curves indicated as in Fig. 456.

bodies, on injection into the blood stream, cause an increased flow of lymph from the thoracic duct (Fig. 456). They may be extracted from the dried tissues of crayfish, mussels, or leeches by simple boiling with water. Commercial peptone has a similar effect. Their effect on lymph production is probably due simply to their deleterious action on the capillary wall. Although these bodies act chiefly on the liver capillaries, they can be shown also to have some effect on the intestinal and skin capillaries. In fact, the injection or ingestion of these bodies often gives rise to a copious eruption of nettlerash due to an increased exudation of tissue fluid into the meshes of the cutis. Histamine, which is present in most tissue extracts, has a similar effect.

An increased lymph flow from the thoracic duct may be produced also by the injection of large amounts (10 to 40 grammes) of innocuous crystalloids, such as glucose, urea, or sodium chloride, into the circulation. In this case the lymph becomes much more dilute. The explanation of the action of these bodies is very simple. We have already seen that after the injection of large amounts of glucose into the circulating blood, a condition of hydræmic plethora is brought about. This increase in the total volume of the blood has two consequences; first, the colloidal osmotic pressure of the plasma proteins is lowered owing to dilution, and second, there is an increased capillary pressure (Fig. 457, A). That the action of these bodies is purely mechanical is shown by the fact that, if the rise of capillary pressure be prevented by bleeding the animal immediately before the injection, the increase in the lymph flow is also prevented (Fig. 457, B).

MOVEMENT OF LYMPH. In the frog, the circulation of lymph is maintained by rhythmically contracting muscular sacs. In the higher animals, the onward flow of lymph is effected partly by the pressure at which it is passed out into the lymph capillaries, and by pulsations of nearby arteries, but also to a large extent by the contractions of the skeletal

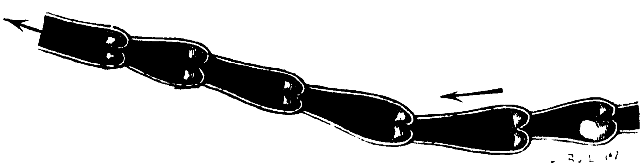

FIG. 458 A Lymphatic Vessel laid open to show Arrangement of the Valves. (TESTUT.)

muscles. In the smaller lymph radicles the pressure of lymph may attain 8 to 10 mm. of water. In the thoracic duct, at the point where it opens into the great veins of the neck, the pressure is obviously about the same as in these veins, that is to say, from − 4 to 0 mm. Hg, the negative pressure being occasioned by the aspiration of the thorax. This difference of pressure is sufficient to cause a certain amount of flow. It must be remembered, however, that under normal circumstances no lymph at all flows from a resting limb. The only part of the body which gives a continuous stream of lymph during rest is the alimentary canal, the lymph of which is poured out into the lacteals, and thence makes its way through the thoracic duct. Movement, active or passive, of the limbs at once causes a flow of lymph from them.* Since the lymphatics are all provided with valves (Fig. 458), the effect of external pressure on them is to cause the lymph to flow in one direction only, viz. towards the thoracic duct and great veins. Hence we may look upon muscular exercise as the greatest factor in the circulation of lymph. The

* WHITE, FIELD and DRINKER. *Amer. J. Physiol.*, 1933, **103**, 34.

flow of lymph from the commencement of the thoracic duct in the abdominal cavity to the main part of it in the thoracic cavity is materially aided by the respiratory movements; since, with every inspiration, the lacteals and abdominal part of the duct are subjected to a positive pressure, and the intrathoracic part of the duct to a negative pressure.

Lymph Glands. Where a lymphatic gland is interpolated on the lymph path, a number of afferent lymph vessels enter it, and discharge the lymph into cortical lymph channels. After percolating through the gland the lymph is collected from the medullary part by a single efferent vessel; the emergent lymph contains many more lymphocytes than were present in the afferent stream.

Lymphatic glands serve to collect particles, and inactivate bacteria and toxins carried to them in the lymph stream: this is effected by the lymphocytes and monocytes in the gland, and it is often found that when tissues become infected with micro-organisms, the up-stream lymph glands become swollen and painful.

If a lymph gland is partially excised, the tissue regenerates, but after complete removal there is no re-formation of the gland. Owing to the ease with which new lymphatic vessels are formed, the removal of even a considerable number of lymphatic glands in an area is not usually followed by lymphatic obstruction.

THE ABSORPTION OF LYMPH AND TISSUE FLUIDS.* On injecting a coloured solution, or a fine suspension, into the connective tissues of any part of the body, and gently kneading the part, it is found that after a time the fluid fills all the lymphatic channels running from the part; and we can in this way inject the lymphatics of the limb, and trace their course on to the thoracic duct. The same path is taken by micro-organisms as they spread in the tissues, or by particles of carmine or Indian ink which have been introduced in tattooing.

This process of lymphatic absorption is a slow one, unless aided by passive or active movements of the surrounding parts, and cannot therefore account for the rapid symptoms of poisoning which supervene within two or three minutes after the hypodermic injection of a solution of strychnine or other poison. That such absorption is not dependent on the lymphatics is shown by the fact that the symptoms occur almost as quickly when all the tissues of an infected limb have been severed, with the exception of the main artery and vein. In the absorption of fluids from the pleural and peritoneal cavities, movements, such as those of respiration, play a part by aspirating fluids into the lymphatic capillaries. Yet, after injecting methylene blue or indigo carmine into the pleural cavity or subcutaneous tissues, the dyestuff appears in the urine long before any trace of colour can be perceived in the lymph flowing from the thoracic duct. The absorption in these cases is by the blood capillaries, and consists in an interchange between blood and extravascular fluids, by diffusion.

Definite, though slow, passage of fluid, of colloidal dyes, of toxins and even of bacteria has been shown to occur from the nasal passages into the cervical lymph, especially if passive movements of the head are made.

More difficulty is presented by the question of the mechanism of absorption, into the blood vessels, of the normal tissue fluids—such an absorption as we have seen to occur after loss of blood by hæmorrhage. It seems probable that this absorption depends on the small proportion of protein contained in the tissue fluid as compared with the blood plasma, and is due to the osmotic

* STARLING and TUBBY. *J. Physiol.*, 1894, **16**, 140; STARLING. *J. Physiol.*, 1896, **19**, 898, **22**, *Proc.*, xxiv.

pressure of the protein. Thus, we may conceive that there is normally a balance in the capillaries between the processes of exudation and of absorption, the former being conditioned by the capillary blood pressure and the latter by the difference in protein content, and therefore of colloidal osmotic pressure, between the blood plasma and tissue fluid. A rise of capillary pressure will upset this balance in favour of transudation, and the blood will become more concentrated; whereas a fall of pressure will turn the scale in favour of absorption, and the volume of blood will be increased at the expense of the tissue fluids.

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THE CEREBRO-SPINAL FLUID

Four membranes are interposed between the bony wall of the cranial cavity and the brain itself. From outside inwards, these are: (1) the periosteal layer of the dura; (2) the meningeal layer of the dura; (3) the arachnoid, and (4) the pia. The cerebro-spinal fluid lies in the space between the arachnoid and the pia.

The dura consists of firm fibrous tissue; its outer part represents the periosteum, and this is firmly attached to the interior of the cranium. Fibrous partitions are sent from the dura into the cavity of the cranium, thus supporting the chief parts of the brain. One of these, the falx cerebri, lies between the two cerebral hemispheres. A second, the tentorium cerebelli, forms a horizontal division between the cerebral hemispheres and the cerebellum. A third one, the falx cerebelli, passes a short distance in between the cerebellar hemispheres. In the spinal canal, the bones have their own periosteum, and the meningeal dura, which is closely attached round the margins of the foramen magnum, forms a loose sheath round the spinal cord. Tubular prolongations are formed where each nerve-root emerges, and are continuous with the outer sheaths of the nerves. Where the venous sinuses occur, the two layers of the dura separate, so that the venous sinus lies between them. These sinuses are angular clefts, the chief of which lie along the attached margins of the falx cerebri and the tentorium cerebelli. The greater part of the blood which these contain drains into the internal jugular veins. In the spinal cord, these venous sinuses are replaced by plexuses of thin-walled veins, which are embedded in fat and lie on the outside of the dura. The arachnoid is a delicate, transparent membrane, covering the whole of the brain and spinal cord. Superficially it is covered by a layer of endothelial cells which bounds the sub-dural space. On its deep surface, it is connected with the pia mater by numerous fine fibres. It bridges over the inequalities in the surface of the brain, so that spaces are left which are filled with cerebro-spinal fluid. The arachnoid surrounds the cerebral and spinal nerves, and encloses them in loose sheaths as far as their points of exit from the skull or vertebral canal. On the upper surface of the brain it is very thin; at the base it is thicker. The spinal part of the arachnoid is thin and delicate. It consists histologically of white fibrous and elastic tissue intimately blended, and contains blood vessels and a rich plexus of nerves. Wherever it comes in contact with cerebro-spinal fluid it is covered by flattened polygonal mesothelial cells.

There is a considerable space between the arachnoid and the pia covering the spina cord, and since the cord ends at the first lumbar vertebra, whereas the dura and arachnoid extend to the second sacral vertebra, it is easy to puncture the theca and so to draw off cerebro-spinal fluid, by means of a hollow needle. This simple operation, called *lumbar puncture*, is usually performed by piercing with the needle at the interval between the

fourth and fifth lumbar vertebræ, and is of considerable value for diagnostic purposes as well as for the induction of "spinal anæsthesia," by the introduction of appropriate local anæsthetics into the thecal space.

The pia mater is a layer of connective tissue which serves to carry the blood supply to the whole surface of the brain. It closely follows all the irregularities of the latter, dipping down into the fissures and sulci of the brain. In the spinal canal, the pia sends out a series of processes, the ligamentum denticulatum. The outer extremities of these are attached to the dura mater, and help to sling the spinal cord in its dural sheath. By means of the rich blood supply conveyed by the pia mater, the brain is abundantly supplied with blood. The two carotid and two vertebral arteries convey the blood into the cranial cavity. The vertebrals unite to form the basilar artery, which divides again into two branches, and these unite with branches of the internal carotid arteries to form the circle of Willis. From this circle, three main arteries on each side pass to supply separate regions of the brain, two anterior, two middle, and two posterior cerebral. The inner parts of the brain, *e.g.* corpus striatum, are supplied by branches of the circle of Willis which pass straight into the brain substance. The anastomosis between branches appears to be very slight. In consequence, if any one vessel becomes obstructed, the blood supply of a considerable brain area may be cut off.

THE SUB-ARACHNOID CISTERNÆ. The sub-arachnoid cavity, which is the space between the arachnoid and pia, contains the cerebro-spinal fluid and the larger blood vessels of the brain. It is traversed, as we have said, by spongy tissue consisting of delicate trabeculæ, which run from arachnoid to pia. In most parts of the cranial cavity, the space thus formed is small; in certain parts near the base of the brain, however, the arachnoid is separated from the pia by wide intervals which communicate freely with one another and are named the sub-arachnoid cisternæ, *e.g.* the *cisterna magna*, below the cerebellum. The whole of these cavities, which contain the cerebro-spinal fluid, are lined by flattened mesothelial cells.

THE CHOROID PLEXUSES. These plexuses, which are found lying in the ventricles of the brain, consist of highly vascular tufts which are covered by ependymal cells. These peculiar masses of tissue apparently form a good deal of the cerebro-spinal fluid. Evidence of the secretory nature of the choroid plexuses is partly of a histological, and partly of an experimental, nature. The cells are seen to contain granules, both eosinophil and basophil. Pilocarpine, which generally stimulates glands into activity, is found to exert a pronounced effect on the ependymal cells, so that the protoplasm of the cell becomes greatly increased in amount. The experimental evidence is that if, by blocking the foramen of Monro, one of the ventricles is prevented from getting rid of any fluid secreted into it, then hydrocephalus develops in that particular ventricle. Such a process does not take place if the choroid plexus be first removed from the ventricle.* Normally there is a constant flow of fluid from the lateral ventricles, so that it can be drawn off continuously by a catheter passed through the aqueduct of Sylvius into the third ventricle.†

Doubtless the rate of formation of cerebrospinal fluid is accelerated by any circumstance which produces an increase of capillary pressure in the choroid plexuses. For example, pressure may be exerted on the vein of Galen by reason of the vascular engorgement of a small body, the suprapineal arachnoid body,‡ possibly under nervous control, and thereby the capillary pressure in the third and lateral ventricles may be increaesd.

THE ARACHNOID VILLI. These are minute processes of the arachnoid which are found to project into the venous sinuses. Where these projections occur, the dura mater is interrupted. The processes themselves are lined by two layers of cells. Externally they are covered by the

* DANDY *Trans. Amer. Surg. Assoc.*, 1919, 37, 397.
† WEED. *J. Med. Res.*, 1914, 26, 93.
‡ LE GROS CLARK. *B.M.J.*, 1940 (i), 476.

endothelium of the venous sinuses, and internally by the mesothelial cells of the arachnoid mater. They are present in most animals, as well as in men of all ages. They are present in most of the cerebral sinuses, and are occasionally found penetrating the dura in other regions.

THE PERIVASCULAR SPACES. Surrounding each of the blood vessels which penetrates into the surface of the central nervous system, is a sleeve formed of mesothelial cells. These tubes are called perivascular spaces. Thus a path is left clear for fluid to escape from the cell spaces inside the brain substance into the cerebro-spinal space. If these perivascular channels are

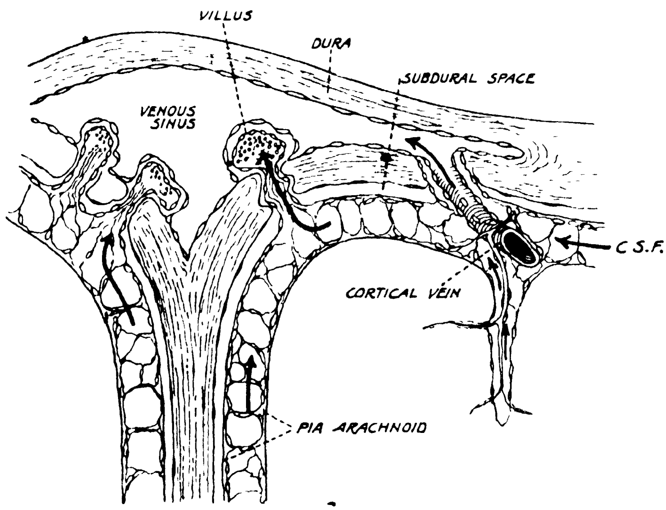

FIG. 459. Diagram of Relations of Sub-arachnoid Space to Arachnoid Villi, Cortical Veins, and Venous Sinus. (After WEED and CUSHING.)

followed inwards, they terminate in innumerable spaces, called perineuronal spaces in which lie the nerve cells.

The relations of the perivascular spaces and arachnoid villi to the cerebro-spinal fluid and the blood are shown in Fig. 459.

THE CEREBRO-SPINAL FLUID. The chemical composition of this fluid is rather like that of lymph. Its specific gravity is about 1005. It contains salts, glucose and a little protein. Urea and creatinine are present in small amounts. Although at first sight the cerebro-spinal fluid might appear to be an ultra filtrate or a dialysate of blood plasma, the balance of opinion seems to be that in its detailed composition it differs materially from those and must be regarded as a true secretion. Its total quantity in man is about 150 c.c. Its pressure inside the cranial cavity is about 100 mm. of water in recumbent subjects, but varies with posture; in the erect posture it may be below the atmospheric pressure. In disease, changes in the chemical composition of the cerebro-spinal fluid may take place. The protein content may increase, and cells may appear in large numbers. When the infections are tuberculous, the cells are principally lymphocytes; in other types of infection they are principally polymorphonuclear leucocytes. In renal disease, chlorides are increased.

Circulation of Cerebro-spinal Fluid. This originates from two sources, the choroid plexuses in the ventricles, and the perivascular spaces found in relationship with the vessels, especially the arterial capillaries of the pia. It flows from the ventricles by way of the foramen of Monro into

the third ventricle, and thence, by the aqueduct of Sylvius, it reaches the fourth ventricle, from which it escapes, by way of the foramina of Majendie and Luschka, into the subarachnoid space. Having originated in this manner, the cerebro-spinal fluid passes slowly towards the convexity of the cerebrum to reach the arachnoid villi, through the walls of which it filters into the venous sinuses. That this is the normal direction taken by the circulation has been amply proved by Weed.* He injected into the lumbar subarachnoid space in animals a mixture of potassium ferrocyanide and ferric ammonium citrate, and, after an interval of several hours, fixed the entire brain in an acid fixative by which Prussian blue was precipitated wherever the mixture had travelled. The coloured precipitate was found all along the subarachnoid space and thence through the arachnoidal villi into the venous sinuses. There is no reason to suppose that the drainage is anything more than a filtration of the cerebro-spinal fluid from the subarachnoid space, where its pressure is higher, into the venous sinus, where its pressure is lower.

Under certain circumstances, it is possible to reverse the normal direction of flow. Instead of cerebro-spinal fluid being produced in the peri-vascular spaces, as is normally the case, in cerebral anæmia the spaces may be widely dilated and absorption of cerebro-spinal fluid may take place there, presumably into the capillaries and veins. Hence, if the ferrocyanide mixture be injected under these conditions, staining of the perivascular spaces, and even of the perineuronal spaces, results. A similar result follows after the intravenous injection of hypertonic salt solutions,† by which water is rapidly abstracted from the brain tissue as well as from the cerebro-spinal fluid, by way of the perivascular spaces. Owing to the rapid osmotic removal of water, the brain also shrinks appreciably, a fact which has been utilized in the performance of surgical operations on the brain, parts of which become much more readily accessible after a preliminary injection of strong salt solution. The total volume of cerebro-spinal fluid is augmented to keep pace with the shrinkage of the brain.

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* WEED. *J. Med Res.*, 1914, **26**, 21, 51, 93.

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CHAPTER XXXVIII

THE DEFENCE OF THE ORGANISM AGAINST INFECTION

THE CELLULAR MECHANISMS OF DEFENCE

IN most cases, invasion of a higher animal or plant by some lower organism is fraught with danger to the host, so that special mechanisms have to be provided for the protection of the tissues from infection. There are four types of lower organism against which defence is needed, viz. *viruses* (*e.g.* measles, small-pox), *bacteria* (*e.g.* tetanus, diphtheria), *moulds* (*e.g.* ringworm), and *protozoa* (*e.g.* malaria, syphilis, amœbic dysentery).

The prevention of infection is of course the function of the epithelial covering, either of the skin, or of the surface of the gut. Protection here may be of a physical or chemical character. The cells may secrete a horny or chitinous layer, which presents a mechanical obstruction to the entry of bacteria. They may secrete mucin, which entangles invading micro-organisms, or they may secrete substances which actually kill such organisms.

The most primitive means of defence, and one which is found throughout the whole animal kingdom, is exactly analogous to the process by which the amœba destroys and utilises any bacteria present in its environment. When a micro-organism has obtained entrance to the interior of the body, *e.g.* through a wound of the surface epithelium, the task of dealing with the invader becomes the office of a special type of cells called *phagocytes*, and the whole process by which particulate foreign material, or the animal's own dead tissues are got rid of, is spoken of as *phagocytosis*. The process of phagocytosis may be studied in its simplest form by injuring or infecting some tissue which is free from blood vessels. Thus, the tail fin of an embryonic axolotl may be cauterised with silver nitrate, or a small quantity of fluid containing carmine granules may be introduced by means of a hypodermic syringe. In either way a certain number of cells are destroyed, and the dead tissue thereupon is treated as a foreign body. As a result, the wandering cells or histiocytes (macrophages) move from the surrounding tissues towards the seat of the injury, and the day after the injury has been inflicted, a collection of these macrophages can be seen, many of which contain particles of carmine, or débris of the destroyed tissue, which they have taken up. The cells finally wander away from the part, and the destruction is made good by the proliferation of the fibroblasts, and of the epithelium immediately adjoining the injury.

By the vascular system, all the tissues of the body are brought into material relationship with one another, so that many distant parts may be drawn upon to supply the needs of any one part. It is evident that the accumulation of cells for the defence of the organism against invading microbes will be much more effective if the blood vessels participate in the process, so that, by their means, the phagocytic resources of all parts

of the body can be drawn upon to ward off a localised attack. The process of phagocytosis thus, in the higher animals, becomes merged into the more complex series of phenomena to which the term '*inflammation*' has been applied. This process can be studied by observing the effects of slight injury to some vascular transparent part of the body, *e.g.* the frog's tongue or mesentery, or the web of the frog's foot. For this purpose a small piece of the skin of the frog's web may be snipped off with fine curved scissors, the section being sufficiently deep to remove the skin without causing hæmorrhage. The first effect noticed in the immediate neighbourhood of the injury is a dilatation of the vessels, especially of the venules, and an acceleration of the blood flow. In the course of an hour, the capillaries also become dilated, and many capillary channels, previously invisible, are now occupied with blood. In some instances, in higher animals, new capillaries have been observed, under the microscope, to grow out from other capillaries, towards the area of inflammation. Through the dilated capillaries there is a rapid blood stream, the corpuscles occupying the axis of the vessel, so that there is a periaxial layer of plasma. A little later, this acceleration gives place to a slowing of the blood stream, and simultaneously the leucocytes of the blood are seen to be adherent to the capillary wall. Apparently the latter becomes what we may call 'sticky,' the effect of the stickiness being to increase the resistance to the passage of the blood through the vessel, and also to cause the adhesion of the leucocytes to the wall. As the current becomes still slower, the distinction between axial and peripheral streams disappears. The corpuscles are now closely packed together, the white corpuscles being predominant at the margins of the capillary, where they form a lining to the vessel (Fig. 460). The next stage is the emigration of the leucocytes. These may be observed to thrust a process through the vessel wall (according to Arnold this process of emigration always occurs through the stigmata, *i.e.* the points where the endothelial cells come in contact—Fig. 461). The prolongation enlarges on the outer side of the vessel, while the portion of the leucocyte within the vessel becomes smaller, so that finally the whole leucocyte passes through, and lies in the tissue spaces outside the capillary. In the course of five or six hours, all the capillaries and small veins in the neighbourhood of the injury may show a crowd of polymorphonuclear leucocytes along their outer surfaces. In addition, there is a migration of certain tissue cells, viz. fibroblasts and histiocytes, to the affected place; later, there is often a further emigration from the blood of histiocytes and of lymphocytes. Of these various cells, the polymorphonuclear leucocytes and the histiocytes are phagocytic, and help to remove invading particles or organisms, and also later to remove the tissue injured by the primary lesion. As soon as this is effected, regeneration of the injured tissue

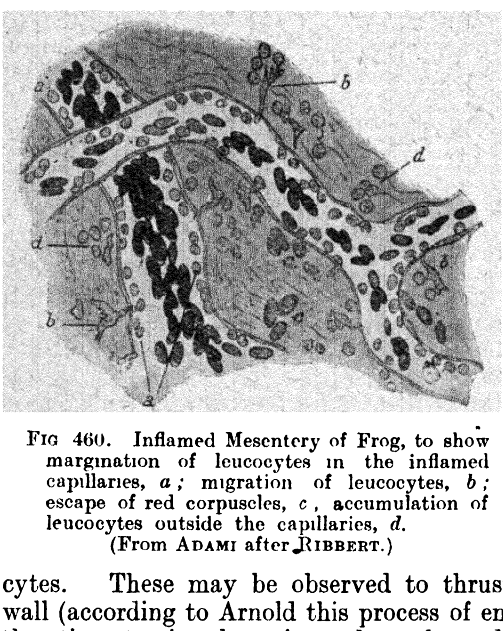

FIG 460. Inflamed Mesentery of Frog, to show margination of leucocytes in the inflamed capillaries, *a*; migration of leucocytes, *b*; escape of red corpuscles, *c*, accumulation of leucocytes outside the capillaries, *d*. (From ADAMI after RIBBERT.)

occurs by a proliferation of the fibroblasts and the epithelium, while the leucocytes are destroyed by macrophages.

Inflammation in warm-blooded animals thus gives rise to dilatation of vessels and increased vascularity of the part, to alteration of the vessel wall and therefore to increased effusion of fluid. There are warmth and redness of the part from the vascular dilatation, swelling from the effusion of tissue fluid, and very often, as a result of the injury or the swelling and the consequent involvement of sensory nerves, pain. The four cardinal symptoms of inflammation, namely, *rubor*, *calor*, *turgor*, and *dolor*, which have been described for generations as typical of this condition, naturally leave out of account the phenomenon of phagocytosis round the seat of injury with the objects of removing injured tissue, of destroying micro-organisms, of protecting the body from general infection, and of preparing the way for reintegration of tissue.

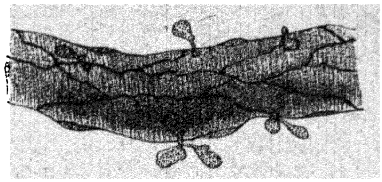

FIG. 461. Emigration of Leucocytes through Capillary Wall. (ARNOLD.)

The essential phagocytic character of the inflammatory process is clearly shown if the primary lesion be attended with infection. Thus, if a small quantity of a culture of staphylococcus be injected into the subcutaneous tissue of the rabbit, the vessels surrounding the point of injection may, within four hours, be found densely filled with corpuscles. In ten hours' time the leucocytes are present in large numbers outside the vessels, while the injected cocci have spread for some distance along the lymphatic spaces and, while still partly free, have been to a large extent ingested by the leucocytes. In twenty hours' time the connective tissue fibrils at the point of injection are found to be widely separated by the aggregation of phagocytes. In forty-eight hours' time a well-defined abscess is produced. At the centre of this, all traces of previous connective tissue have disappeared and its place has been taken by *pus*, *i.e.* a mass of leucocytes, many of them dead and in a state of degeneration, mingled with staphylococci, partly within, partly outside the cells The margin of the abscess is formed by connective tissue infiltrated with living macrophages. A certain number of cocci are to be seen free in the tissue outside this layer, but in the course of a day or two these free cocci disappear, and there is thus a continuous layer of phagocytes surrounding the abscess cavity, and preventing any further invasion of the body as a whole from the seat of infection. The abscess subsequently discharges on to the exterior by a process of necrosis of the overlying skin, and regeneration of tissue takes place in the same manner as in the more trivial injury.

We have abundant evidence to show that the essential factor in this aggregation of leucocytes is their chemical sensibility, and that the phenomenon is simply one of *chemiotaxis*. A capillary glass tube containing a suspension of dead micrococci, or peptone, or broth extracted from dead tissue, if introduced into the anterior chamber of the eye or into the subcutaneous tissue, is found after a short time to be full of leucocytes. We must assume that the chemical products diffusing out of the ends of the capillary tube have occasioned a positive chemiotaxis on the part of the leucocytes. It is worthy of note that the positive chemiotactic influence exerted by any given species of pathogenic bacterium is roughly inversely proportional to its virulence. A culture lacking in virulence may cause a very pronounced aggregation of leucocytes which speedily ingest and destroy the micro-organism, whereas if a culture of a more virulent variety of the same microbe be injected, there

may be all the signs of inflammation, swelling, and large effusion of fluid, though the tissues may contain very few leucocytes. Under the circumstances, the micro-organism rapidly proliferates and spreads from the seat of the lesion, giving rise finally to general infection. Usually, however, when bacteria enter the blood stream, they are rapidly removed by fixed histiocytes of liver and spleen, by polymorphonuclear cells in the blood, and by macrophages in the lungs.

There is no doubt that, in all active inflammations, the polymorphonuclear leucocyte is the form which is attracted first, and in largest numbers, to the seat of injury. It is the characteristic cell from which pus is formed, and is actively phagocytic. It has nothing to do with the regeneration of the destroyed tissue. The eosinophile corpuscle is also present at an early stage around the inflammatory focus, but is never present in numbers at all comparable with those of the polymorphonuclear leucocyte. It is especially abundant in chronic inflammations of certain tissues, such as the skin, but is rarely seen to ingest bacteria, and therefore cannot be spoken of as phagocytic. The lymphocytes predominate in certain chronic inflammations, especially in those caused by the tubercle bacillus. They do not ingest bacteria. The histogenous wandering cells (macrophages) appear in the inflammatory area at a later period than the polymorphonuclear and eosinophile cells. They are actively phagocytic and motile. As a rule, their phagocytic properties are exerted, not on bacteria, but on other cells and cell *débris*. After an acute inflammation, their chief office is to clear away the remains of the polymorphonuclear leucocytes and dead tissues, so as to prepare the way for subsequent regeneration. The fibroblasts are connective tissue cells, and it is held by some investigators that they can be converted into macrophages, and *vice versâ*. It is, however, difficult to be certain whether the wandering and the fixed connective tissue corpuscles are of identical or of different origin. In the process of repair, the fibroblasts are converted into fibrous tissue.

We thus see that several types of the wandering cells of mesodermal origin, which take part in inflammation, do not exert active phagocytic properties and cannot therefore destroy bacteria or other invading organisms by the process of ingestion and digestion. Yet we have evidence that the part played by such cells in the defence of the organism is no less important than that of the actively phagocytic cells. Leucocytes are able to destroy bacteria, not only by the process of intracellular digestion, but also by the excretion, into the surrounding body-fluids, of substances which have a deleterious influence on bacteria. Thus, normal blood serum is found to have a strong destructive influence on most species of bacteria, whether pathogenic or not. Since this property is not shared to anything like the same extent by the blood plasma, it may be ascribed to changes in the leucocytes in the process of clotting and the consequent liberation of bactericidal substances. Many species of pathogenic bacteria cannot infect the animal as a whole. These, nevertheless, may multiply on the surface of the body, or in an abscess cavity, and lead to the death of the host, in consequence of the production by the bacteria of soluble *toxins*, which are absorbed into the blood stream. Examples of such micro-organisms are those which are associated with tetanus and diphtheria. There must be other mechanisms at the disposal of the body for the neutralisation of these toxins. The protection of the body against destruction by bacterial toxins involves in fact a whole series of chemical mechanisms, which we must regard as of equal importance and as co-operating with the phagocytic mechanism.

THE CHEMICAL MECHANISMS OF DEFENCE

INFECTION AND IMMUNITY. All infectious diseases are caused by the agency of micro-organisms. The greater number of these are bacteria or viruses, a certain number must be classed with the yeasts, while others are protozoal in character.

In the protozoal diseases the micro-organisms exist for the greater part as intracellular parasites. One attack of the disease does not as a rule confer immunity, and the treatment has to be sought along the lines of medication by drugs, which will either directly destroy the infecting agent, or enable the tissue to combat it.

The infections due to the viruses are in many ways similar to those induced by bacteria, but in this case the causative organism is so minute that it can pass through the pores of the finest filter, and is invisible under the microscope. They are probably proteins of enzyme-like properties, and cannot be cultivated except in living tissues,* whereas the pathogenic micro-organisms can, usually, be cultivated on artificial media outside the body, and may be divided into two classes.

It is especially in the first class of diseases, namely, those due to bacteria, that the organism has developed chemical mechanisms of defence. The diseases due to bacteria include diphtheria, tetanus, tubercle, anthrax, typhoid and many others. In these diseases we have to deal with a number of phenomena more or less common to all. The infection in each case is due to the actual transference of the specific organism from one animal to another. After the micro-organism has attained entrance into the system there is a period of incubation before the disease actually breaks out. When this occurs, the specific microbe is to be found in large quantities either in the blood or in the tissues of the body. The disease is generally characterised by fever and often by local lesions, such as the intestinal ulcers of typhoid, or the glandular swellings of bubonic plague. The micro-organisms may develop in the animal until its death, or the disease may terminate in recovery and the total disappearance of the microbes from the body. Some animals or individuals are naturally immune to particular diseases, *e.g.* dogs to typhoid, and this is called *natural congenital immunity*. In other cases, it is found that, after recovery, the patient cannot be reinfected by the bacterium which was the cause of the disease, and this condition, called *natural acquired immunity*, may last as long as the patient lives.

Toxins. One class, of which the diphtheria and tetanus bacilli are examples, secrete into the surrounding culture-fluid substances, which, when injected into animals, act as virulent poisons. The other class of bacteria do not form such extracellular toxins, but in their case it is found that, if the bodies of the bacilli be broken up, the injection of the contents of the bacteria is attended with poisonous effects. The bacteria may be thus classified according as they produce *extracellular* or *intracellular toxins*.

We may first deal with the manner in which the body reacts to the exotoxins. If a culture of diphtheria or tetanus bacilli be filtered, the clear filtrate free from bacilli is found to exercise as poisonous results as if the culture of the living bacilli had itself been employed. The toxins contained in these fluids are extremely potent. Thus five-millionths of a gramme of dried tetanus toxin is a fatal dose for a mouse, and 0·00023 gramme would kill a man; the pure toxins, which have never been isolated, must be much more powerful. The

* STEINHARD and LAMBERT. *J. Infect. Dis.*, 1914, **14**, 87; MAITLAND and MAITLAND, *Lancet*, 1928 (ii.), 596.

toxin is an unstable body, and is destroyed by heating to 65° C. The nature of toxins is not known, though they probably contain proteins. Similar toxins are widely distributed. Thus they form the active constituent of snake venom and of the poison of scorpions and spiders. They also occur in the seeds of castor oil and of jequirity, the toxins of which are proteins, known respectively as *ricin* and *abrin*. There is a great variability in the reaction of different animals to these toxins. To the poison of tetanus, the rabbit is, weight for weight, two thousand times, and the hen twenty thousand times, more resistant than the guinea-pig. A certain time is necessary after the introduction of the toxin before its effects are displayed. There is a striking difference in this respect between the action of these complex bodies and the action of drugs, such as strychnine or morphine. This time cannot be reduced beyond a certain limit, however much toxin be injected. Thus, a lethal dose of diphtheria toxin kills a guinea-pig in fifteen hours. If ninety thousand such doses be injected into a guinea-pig, it is not possible to reduce the time of survival below twelve hours. Another characteristic of these toxins is the specificity of their action. One kind of toxin may act chiefly on the central nervous system, another on the peripheral nerves, another on the red blood corpuscles. Associated with this specific action is the actual combination which occurs between the toxin and the organ on which it exerts its effect. Thus, tetanus toxin has a specific affinity for the central nervous system, and may be removed from a solution by shaking the latter with an emulsion of the brain of a susceptible animal, such as a guinea-pig, but not with one of the brain of an animal of a non-susceptible species.

Antitoxins. In spite of the excessively fatal character of these toxins, it is possible to render an animal immune to their action. If a dose of diphtheria or tetanus toxin which is smaller than the fatal dose be injected into an animal, the latter shows signs of injury, from which it recovers. When recovery is complete, it is found that three or four times the fatal dose may be injected without producing any evil effects; and this process of injection of toxin may be repeated in continually increasing doses, until the animal may finally be able to withstand a dose one hundred thousand times as large as that which would have been fatal to it in the first instance. This is a condition called *active artificial immunity*, and it is found that the blood serum of the immunised animal has the power of neutralising the toxin. Thus, if blood serum from a horse which has been treated with large doses of diphtheria toxin be mixed with an equal quantity of the toxin itself, the mixture may be injected into susceptible animals without the production of any effect. It is possible in this way to get a serum, 1 c.c. of which will neutralise many fatal doses of the toxin; further, this antitoxic serum may be injected into a susceptible animal and then confers *passive artificial immunity* on the latter, or it may be injected into a diseased animal and so used as a curative agent. *Antitoxins* thus play a great part in therapeutics, especially of tetanus and diphtheria. In the case of tetanus, the toxin appears to be absorbed by the motor end plates, from which it diffuses along the motor axons to reach the ventral horn cells of the cord. By the time that it has arrived at the central nervous system, and the spasms typical of tetanus have broken out, the toxin is already so firmly bound to the reacting tissue that the injection of antitoxin into the blood stream has little or no effect on the course of the disorder. The use of the tetanus antitoxin is, therefore, chiefly as a prophylactic agent.

If we define a unit of toxin as that amount which possesses a certain power, *e.g.* which will kill a guinea-pig in so many days, or will cause the complete hæmolysis of

1 c.c. of blood in two and a half hours, we can find the amount of anti-body which is just sufficient to neutralise this effect, and this amount of anti-body can be regarded also as one unit. If, instead of one unit of each, we take 100 units, the neutralisation is effected in the same way. The process is found, however, to be more complex when we take 100 units of toxin, or lysin, and attempt to neutralise them by the fractional addition of antitoxin. In the case of a strong acid and strong alkali, we know that if 100 c.c. of alkali are just sufficient to neutralise 100 c.c. of acid, the addition of 50 c.c. of alkali will leave half the acid unneutralised. If we try the same experiment in the case of mixtures of toxin and antitoxin, it will be found that the addition of 50 units of antitoxin will neutralise much more than half of the toxin, and the same applies to other bodies of this class. Further, 1 c.c. of antilysin exactly neutralises 1 c.c. of lysin;

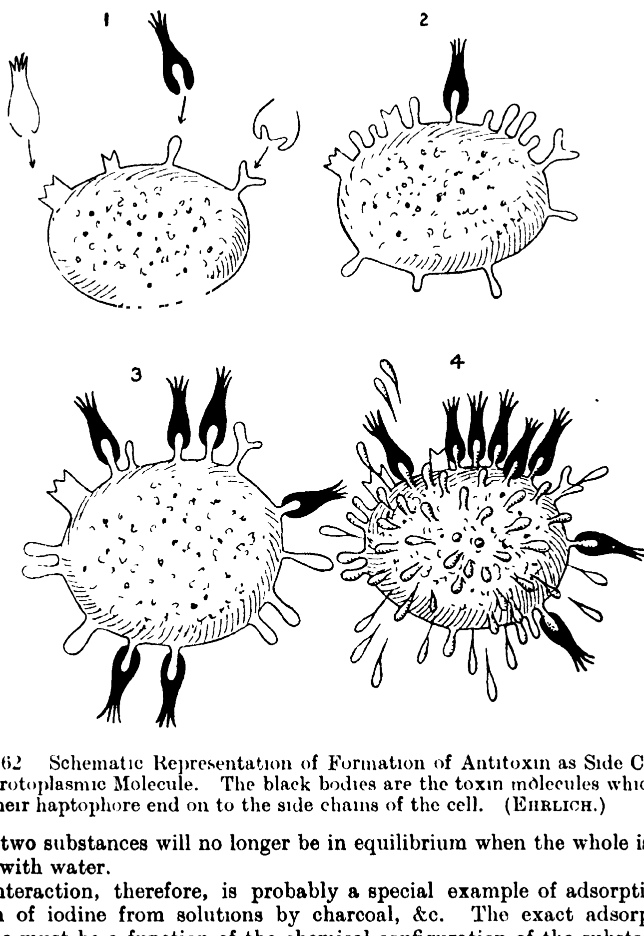

FIG 462 Schematic Representation of Formation of Antitoxin as Side Chains of Protoplasmic Molecule. The black bodies are the toxin molecules which fit by their haptophore end on to the side chains of the cell. (EHRLICH.)

but these two substances will no longer be in equilibrium when the whole is diluted up to 10 c.c. with water.

The interaction, therefore, is probably a special example of adsorption, like the adsorption of iodine from solutions by charcoal, &c. The exact adsorption which takes place must be a function of the chemical configuration of the substance forming the surface, since otherwise it would be impossible to account for the extremely specific character of the interaction between toxins and their corresponding antitoxins. The interaction must, therefore, be assigned to that special class, in which we have already placed the action of enzymes, which is not entirely chemical nor entirely physical, but depends for its existence on a co-operation of both chemical and physical factors. In any case, the antitoxin does not *destroy* the toxin, since it is possible, from an inert mixture of toxin and antitoxin, to separate toxin and anti-body once more.

THE SIDE-CHAIN THEORY. How are we to account for the production, as a result of the injection of toxins into the body, of antitoxin far transcending the amount of toxin injected ? In all the speculations on the mode of production and action of antitoxins an important part has been played by a conception put forward by Ehrlich in 1885.

According to this conception, which is spoken of as the 'side-chain theory,' each unit of living matter consists of a centrally placed portion with a number of side chains attached to it. To these side chains, or *receptors*, various nutritive or other substances may be linked, provided their chemical configuration is of the right kind. Ehrlich regarded the toxins as possessing two side chains, one of which, by its stereomeric configuration, is peculiarly adapted to fit on to the side chains of the protoplasm which the toxin attacks, and this group is known as the *haptophore* group ; and another side chain, the *toxophore* group, which is responsible, when the toxin is once anchored, for the destructive changes wrought by the toxin on the cell of the body (Fig. 462, 2). The antitoxins are supposed to act by uniting with the haptophore group, and so preventing the susceptible protoplasm from doing so and suffering their injurious effects.

The formation of antitoxins is accounted for on this hypothesis in the following manner. When a receptor of the cell is occupied by becoming attached to the haptophore group of the toxin, this side chain is, so to speak, shut out from the normal activities of the cell. A defect is thus produced in the cell, to which the latter endeavours to adapt itself by the production of other side chains of the same character (Fig. 462, 3, 4). It may be regarded as a general rule in living tissues, that a reaction tends to be an over-reaction, so that the compensation by the cell should more than make good the defect produced by the attachment of the toxin. We thus get, not one, but a number of side chains produced, of the same character as that occupied by the toxin molecule, and therefore able also to act as receptors for the haptophore group of the toxin. These new receptor side chains, being produced in excess, are supposed by Erhlich to be thrown off from the cell and to circulate in the body fluids (Fig. 462, 4). A number of protoplasmic fragments are thus set free which have a specific power of uniting with the toxin, and it is this excess of side chains, thrown off from the cell, which represents the antitoxin molecules found circulating in the blood after the injection of toxins. It will be noted that this theory, though chemical in form, is really purely biological. It does not explain the phenomena by reference to the known laws of chemistry, but is a manner of re-naming the biological phenomena, which facilitates their description and discussion and enables us to classify the very complex phenomena of immunity, though only in an imperfect fashion.

A more modern version of the hypothesis is that the antibodies are not formed in all cells of the body, but that the reticulo-endothelial cells, which are known to be active in taking up various antigenic materials, react by producing antibodies.

Antigens. The property of giving rise to anti-bodies on injection into an animal is not confined to toxins ; a large number of substances, all of them containing proteins, have the same property. All such substances are classed together as *antigens*, and are highly specific. Some proteins, *e.g.* insulin, gelatin, cannot act as antigens. Bacteria, alive or dead (and thus including endotoxins), or any animal or vegetable cells, venoms, viruses, or pure proteins derived from any of them, can act as antigens, provided they are foreign to the species of animal into which they are injected. (An exception is the protein of the crystalline lens, which can act as an antigen even for the same species.) They all give rise to the production of *specific anti-bodies.* Thus, human serum injected into a rabbit produces in a few days in the rabbit's serum some body which will give a precipitate when mixed with human serum even in minute traces. This *precipitin* formation is specific, so that it may be used as a test for the origin of any unknown specimen of serum.

The antigenic properties of proteins may be artificially altered by the introduction of new groups : *e.g.* insulin and gelatin become antigenic when coupled with glucosidotyrosine; the resulting antibodies, however, do not react with the same antigen, but with the globulin analogue, glucosidotyrosyl-globulin.*

In a similar way, using an extract of dead bacteria, or any protein, as antigen, a specific precipitin for the bacterial extract or for the particular protein, as the case may be, develops in the blood.

* Clutton, Harington and Yuill. *Bioch. J.*, 1938, 32, 1111.

A *vaccine* is such a collection of killed bacteria or virus, and is often injected into atients to stimulate the production of anti-bodies.

When live foreign cells, *e.g.* bacteria or red cells, have been injected, there is developed n anti-body which causes these cells, when added to the serum, to gather together or gglutinate. Typhoid fever can be diagnosed by the fact that the patient's serum causes gglutination of the bacteria from a pure typhoid culture (Widal test). The anti-body oncerned is called an *agglutinin.*

The amount of an antibody formed after a single injection of an antigen varies with he quantity of antigen given, though not in direct proportion. After the injection, the ntibody begins to appear in a few days, reaches a maximum about the fifteenth day, nd then declines, with diminishing speed, over a period of months or years. Second loses of antigen cause earlier and larger antibody formation.

The nature of these specific anti-bodies, and the cause of their high specificity to the ntigens which give rise to them, are not exactly known. They appear to be specialised erum globulins, possessing specific groups which enable them to unite with the corre-ponding antigens. With regard to the antigens of bacteria, recent work has indicated hat, while some of the specificity is attributable to proteins, the most specific immuno-ogical reactions are often associated with the presence of highly specialised carbohydrates r lipoids, as well as proteins.*

Cytolysins. We have already seen that normal blood serum may xert a paralytic or destructive action on bacteria. Light has been thrown

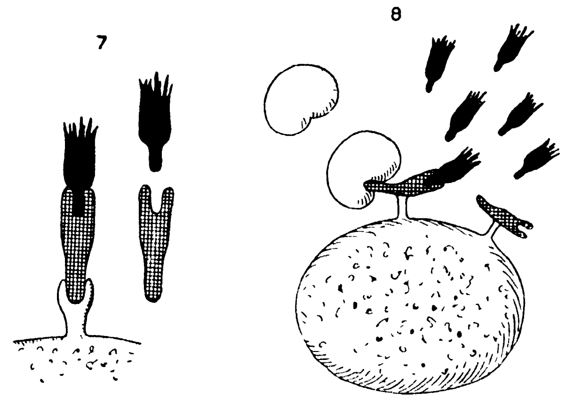

FIG. 463. Diagram to show the relation of Amboceptor and Complement to Red Corpuscles (7) and to the Animal Cell (8). (EHRLICH.)

n the factors involved in this destruction by a study of the phenomena which result when living cells are injected. Normal goat's serum may be nixed with the red blood corpuscles of the sheep without any injury to the atter. If, however, washed sheep's erythrocytes be injected at intervals of a ew days into a goat, the goat's serum is found to have acquired the power f rapidly agglutinating, and then of dissolving, the red blood corpuscles. A pecific *hæmolysin* is said to have been produced. This hæmolytic power can e tested by mixing the serum and the washed blood corpuscles together and llowing the mixture to stand in a narrow tube. The corpuscles rapidly sink o the bottom, leaving the colourless serum above, unless hæmolysis has ccurred, in which case the serum will be of a transparent red colour.

If the hæmolytic serum be heated to 60° C. it is found to have lost its power f dissolving sheep's corpuscles. This power is at once restored if to the eated serum be added any normal blood serum, even of the sheep itself.

* THOMSEN. "Antigens in the Light of Recent Investigations," 1931. (Copenhagen. evin and Munksgaard.)

It seems, therefore, that two substances are involved in the hæmolysis, namely, (*a*) a substance present in most normal sera, and believed to be formed by the leucocytes ; it is destroyed at a temperature of 60° C. and has been called the *complement* ; and (*b*) a substance which is present in the serum only, as a result of the previous injection of some species of red blood corpuscle, which is resistant to the action of heat, and is called the *amboceptor.*

Apparently the amboceptor becomes attached to the red cell first, and then the complement is linked on to this and hæmolysis ensues. Thus, if a rabbit is sensitised against human red cells, and some of its serum taken and heated to 60° C., it will not now hæmolyse human red cells, because the complement has been destroyed by heat. If the human cells added to it are now removed by centrifugalisation, the addition of complement to them, *e.g.* normal guinea-pig serum, causes immediate hæmolysis, whereas if complement is added to the supernatant serum removed from the red cells, no hæmolysis occurs on adding fresh human cells, because the amboceptor has all been removed by those first added.

The function of the amboceptor thus seems to be to enable the complement already present in normal serum to act upon the red blood corpuscles. We may regard the amboceptor, therefore, as having two haptophore groups, one of which anchors on to the red blood corpuscle, while the other attaches itself to the complement (Fig. 463, 7). The amboceptor *plus* the complement thus comes to resemble the toxin molecule, having a free haptophore group at one end and a toxophore group (the complement) at the other end. The reaction to the injection of the red blood corpuscles consists in the formation of the amboceptor, which is essentially the anti-body of the red blood corpuscle (Fig. 463, 8).

Similar specific anti-bodies, capable of effecting the dissolution of cells or organisms, may be produced by the injection of various species of bacteria, or of animal cells, such as leucocytes, spermatozoa, liver cells, &c., and there can be no doubt that bacteriolytic substances play a considerable part in acquired immunity.

A mixture of red cells and specific hæmolytic amboceptor (heated immune serum) can be used as a test for the presence or absence of complement. If complement is added, hæmolysis occurs, as shown above. Based on this fact, and on the further fact that complement is fixed by the amboceptor in presence of its specific antigen, is the important *complement-fixation test* for the identification of either antigens or amboceptors. An important test related to this is the *Wassermann reaction* for syphilis; the patient's serum (= amboceptors ?) is heated to 55° C., mixed with certain lipoids (= antigens ?), and then some guinea-pig serum (complement) added. After a time, heated anti-sheep serum from a rabbit, and washed sheep corpuscles, are added, to test whether complement has been fixed or not. Hæmolysis indicates no fixation, and therefore a negative result of the test; absence of hæmolysis, a positive result.

Opsonins. In some cases the anti-bodies produced by the injection of living or dead micro-organisms do not bring about actual destruction of the bacteria, but alter them in such a way as to make them more susceptible to the action of the phagocytes. If washed white blood corpuscles be mixed with cocci, such as those found in an ordinary boil, they are found to take up the micro-organisms in considerable numbers. The numbers taken up are much increased in the presence of serum derived from an individual who has received repeated vaccine injections of the dead micrococci in question. To the substances in the serum, which thus prepare the micrococci for ingestion by the phagocytes, Wright has given the name of opsonins. The opsonic index of the leucocytes of any individual, in reference to a given species of microbe, is determined by observing the number of the microbes taken up by the leucocytes after treatment with the serum of the individual, and then comparing it with the number taken up by the same leucocytes when the bacteria have been treated with the serum of an average individual.

We thus see that immunity, whether innate or acquired, is extremely complex in character and may depend on one or more of many factors.

The immunity of an animal to any given infection may be determined by the absence from his body of receptor groups for the toxin excreted by the microbe responsible for the infection, or by the fact that the receptor groups, though present, are confined to tissues on which the toxophore group can have no influence. Thus, *e.g.* an attachment of the tetanus toxin to a connective tissue cell would be without effect on the health of the body. Again, immunity may be due to the efficacy of the phagocytes, either of the fluids or of the connective tissues, in ingesting and destroying the micro-organisms, and this, as we have seen, may again be dependent on the presence in, or absence from, the body fluids of substances which, while not destroying the micro-organisms, render them more accessible to the action of the phagocytes. In those cases where the infecting organism secretes a specific toxin, the main line of defence, and the main factor in the production of immunity, is the formation of specific antitoxins to the poison in question. Finally, there may be produced, as a result of the excess of micro-organisms, substances such as the amboceptors, which render the micro-organisms susceptible to destruction by the complements or cytases normally present in the circulating fluids, and possibly themselves derived from the activity or destruction of the leucocytes and other phagocytes of the body.

In this short description we have been able to touch only upon the most salient features of the immunity problem. The question enters strictly into physiology, since, as we have seen, it involves adaptations on the part of the organism to changes in itself or in its environment.

ANAPHYLAXIS (*v.* also p. 58). If an animal be given injections of a foreign protein, it is found that, after ten or more days, a small dose of the same antigen causes a very severe reaction, or death. This, which appears like the opposite to immunity, is called anaphylaxis. Death occurs owing to profound shock, which manifests itself differently in different species; thus in the guinea-pig death is due to asphyxia from constriction of the bronchioles. In the dog death is due to engorgement of the liver from constriction of branches of the hepatic veins. These differences are explained by the differences in the abundance and distribution of plain muscle in different species, for in all cases generalised contraction of plain muscle is the outstanding feature of anaphylactic shock; Dale has shown that the plain muscle of a sensitised animal gives a powerful contraction when a trace of the antigen is added, even if the tissue has been previously washed free of all blood. The reaction is, therefore, due to a direct action of the antigen on the tissues, and there is much evidence in favour of the belief that the substances passed out as 'precipitins' into the plasma are at a later stage absorbed by the tissue cells, and so sensitise these to the antigen. Thus, the anaphylactic sensibility is found to wax as the precipitin content of the serum wanes.

The close similarity between the anaphylactic responses of different tissues in various species of animals, and the responses of those particular tissues to histamine, led to the suggestion that the phenomena of anaphylaxis were due to the liberation of histamine into the blood, and its subsequent distribution all over the body. The fact that histamine can be shown to be produced in the isolated perfused lungs of sensitised guinea-pigs gives support to the idea, but the anaphylactic response of isolated tissues discovered by Dale proves that the dissemination of histamine by the blood stream is only a partial explanation of the phenomena. Lewis observed that the local anaphylactic response in the human skin was similar to the response to other skin injuries, and it now seems probable that the phenomena are due to the liberation, within the cells, of a histamine-like substance, in consequence of the injury produced by the union of anti-body and antigen.

Anaphylactic shock may be met with in medical practice, as when a second dose of a serum is given after a long interval. The name has also been loosely used to describe many different shock-like conditions, some of which are probably due to a direct toxic action on the vascular system, and in which there are no phenomena of antigenic sensitisation. The anaphylactic reaction is highly specific, and the antigens are invariably proteins.

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CHAPTER XXXIX

RESPIRATION

THE MECHANICS OF THE RESPIRATORY MOVEMENTS

IN multicellular animals tissue respiration is carried out between the internal medium, *e.g.* blood, and the individual cells; and the use of the special organ of respiration is to bring the circulating internal medium in intimate relation, over a large area, with the surrounding air or water. In these animals we can distinguish two processes—viz. (1) the interchange of gases between the tissue cells and the surrounding fluid, 'internal respiration'; (2) the interchange of gases between the circulating fluid and the external medium, 'external respiration.' In most water animals the respiratory organ, the gills, presents a large surface, well supplied with blood, over which a continual stream of water is kept up.

In all air-breathing vertebrates the organs of external respiration, the lungs, contain numerous air sacs. The renewal of the air in the air sacs is effected by the movements of respiration, while a rapid circulation of blood is carried out through a fine meshwork of capillaries just underneath the surface of the sacs.

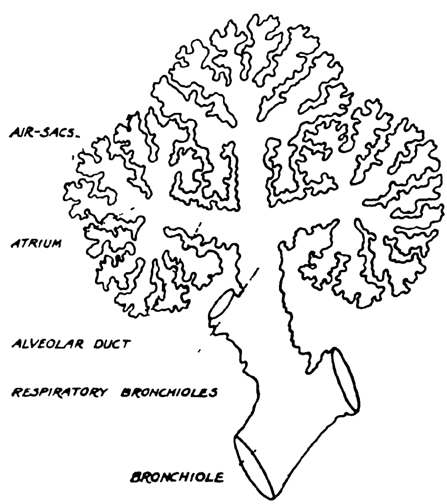

FIG. 464. Diagram of the Air-chambers derived from a Bronchiole. (After MILLER.)

In man the lungs are built up in the following way: The trachea or windpipe, a wide tube about $4\frac{1}{2}$ inches long, gives entrance to the air. It divides below into the two main *bronchi*, and these subdivide again and again into primary and secondary bronchi, &c., each branch becoming smaller, although the total area of their cross-section is together greater than that of the parent tube. The terminal ramifications of the conducting airway, called *bronchioles*, open into *respiratory bronchioles*, and these branch again into *alveolar ducts*.

Confusion having arisen in the use of the terms 'atria' and 'infundibula,' which, in any case, have no particular physiological significance, it is proposed to omit these, and to regard the alveolar ducts as forming a branching network of tubes, opening into *alveolar sacs* or air sacs, in the walls of which are situated minute sacculations, the *alveoli*. A few alveoli also open from the walls of the ducts and respiratory bronchioles (Fig. 464).

The larger tubes are kept patent by incomplete rings of cartilage which are reduced to irregular plates in the smaller tubes and disappear in the bronchioles. The walls contain fibrous and elastic tissue throughout, while in the conducting tubes there is a muscular layer which increases down to the terminal bronchioles, where it is thickest in proportion to the other layers.

Thence it continues, with the elastic tissue, to invest the respiratory portions of the lung, though much reduced in quantity, being broken up by the alveoli which are evaginated through its meshes.

The lining epithelium is ciliated and columnar in the larger tubes, changing to cubical in the respiratory bronchiole, beyond which it becomes flattened to the ' pavement ' type which lines the alveoli.

The walls of the airway are supplied with *arterial blood* by the *bronchial arteries*, and the respiratory parts of the lung receive venous blood from the pulmonary arteries.

The alveoli are the special respiratory parts of the lung. Their thin walls are composed of connective tissue containing a large number of elastic fibres, and are covered internally by a single layer of extremely thin, large, flattened epithelial cells. The alveoli are closely packed together, so that, in a section of the lung, an alveolus is seen to be in contact with others on all sides. The alveoli are not blind sacs, but communicate freely with adjoining alveoli by means of " vents " or stomata in their walls.* These probably serve to equalise air pressures in different alveoli, and to enable the alveoli still to be filled if the duct is occluded.

Immediately outside the pavement epithelium ramify blood-capillaries derived from the pulmonary artery. These form a close network, and the venous blood in them is in proximity to air on two sides, being separated from the air in the alveoli only by the thin endothelial cells of the capillary wall and the flattened cells lining the alveoli.

The lungs in their development grow out from the fore part of the alimentary canal into the front part of the body cavity on each side—the pleural cavity. The surrounding body walls become strengthened by the formation of the ribs, so that the lungs are suspended in a bony cage-work, the thorax. Their outer surface is covered with a special membrane, the *pleura*, which is reflected on to the wall of the thorax from the roots of the lungs, and completely lines the cavity in which they lie. The surface of the pleura facing the pleural cavity is lined with a continuous layer of flattened mesothelial cells, and is kept moist by the secretion of lymph into the cavity. Thus, being attached to the thorax only where the bronchi and great vessels enter, the lungs are able to glide easily over the inner surface of the thorax, with which under normal circumstances they are in intimate contact.

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THE NASAL PASSAGES. The complex nasal passages are covered with a very vascular mucous membrane, by which the incoming air is warmed, and since the mucosa is endowed with mucous glands, the air is also moistened and freed from dust.

It is common knowledge that the nasal passages vary considerably from time to time as regards the ease with which they allow air to enter. Apart from a condition of œdema of the mucosa due to infection, the patency of the nasal passages is subject to change by reason of the variable engorgement of its submucous blood vessels.† The degree of patency may be estimated by blowing a constant stream of air into a nostril, and measuring the pressure of the entering stream ; if the passage is constricted, the pressure is raised, and *vice versâ*.‡ Spraying of the nasal mucosa with adrenaline constricts the vessels and lowers the resistance to air entry ; histamine has the reverse action. Exposure of the body to heat, or to infra-red rays causes closure, while short-

* MACKLIN. *J. Anat.*, 1935, **69**, 188.

† L. HILL. *J. Heat. and Vent. Eng.*, 1934 (March). DUFTON and BEDFORD. *J. Hyg.*, 1933, **33**, 476.

‡ VAN DISHOECK. *J. Hyg.*, 1935, **35**, 185.

waves, or skin-cooling opens the passages. The explanation of the phenomenon is disputed, but it appears to be due to a reflex from the skin. The following table shows the effect of various conditions on the cross-section of the nasal passage, as measured by the rhinomanometric method mentioned above, on a large number of subjects.

AVERAGE CROSS-SECTION IN MM^2 OF NASAL PASSAGES, AT PRESSURE OF 40 CM. WATER (VAN DISHOECK)

Initial cross-section	Electric fire for 10′	Histamine 1 1000	Adrenaline 1 1000
20 8	16 8	10 2	25 1

The effects of stuffy rooms with central heating bears out these observations.

RESPIRATORY MOVEMENTS. A constant renewal of the air in the lungs is secured by movements of the thorax, which constitute normal breathing. The mechanism of these movements was first shown by Mayow in 1668.* With inspiration the cavity of the thorax is enlarged, and the lungs swell up to fill the increased space. The capacity of the air passages of the lungs being thus increased, air is sucked in through the trachea. The movement of inspiration is followed by that of expiration, which causes diminution of the capacity of the thorax and expulsion of air. Expiration normally lasts 1·3 to 1·4 times the length of inspiration. At the end of expiration there is normally a slight pause. The number of respirations in the resting adult is commonly about 17 or 18 a minute. This is, however, much influenced by various conditions of the body, and also by age. Thus a newborn child breathes about 14 times a minute, a child of five about 26 times, a man of twenty-five about 16, and of fifty about 18. The frequency is increased by any muscular effort, even that of standing. These movements are much affected by psychical activity ; they are to a certain extent under the control of the will, although they can occur in an animal deprived of its brain, and are normally carried out without any special act of volition. We can breathe fast or slowly at pleasure, and can even cease breathing for a time. It is impossible, however, to prolong this respiratory standstill for much more than a minute ; the need of breathing becomes imperative, and against our will we are forced to breathe.

With every inspiration, the cavity of the thorax is enlarged in all dimensions, from above downwards by the contraction of the diaphragm, and in its transverse diameters by the movements of the ribs. Quiet breathing is almost entirely diaphragmatic.†

The Diaphragm consists of a central tendon which forms an arched double cupola, to the circumference of which are attached muscle fibres. The diaphragmatic muscles present two main divisions, namely, (1) the spinal or crural part, the fibres of which arise from the upper three or four lumbar vertebræ and from the arcuate ligaments, and are inserted into the posterior margin of the central tendon ; and (2) the sterno-costal part, which arises by a series of digitations from the cartilages and adjoining bony parts of the lower six ribs and from the back of the ensiform process. These latter fibres pass backwards as they ascend. In the cavity of the larger dome on the right side lies the liver, while the smaller dome on the left side is occupied by the spleen and stomach (Fig. 465). These viscera, in the normal condi-

* MAYOW. *De respiratione.* Oxford, 1668.
† HOOVER. *Arch. Int. Med.*, **1922**, **30**, 1.

tion, are pressed against the under surface of the diaphragm by the elasticity of the abdominal walls. The central part of the diaphragm is thus pressed up into the chest, partly by the intra-abdominal pressure, and partly by the elastic traction of the extended lungs. The upper surface of the central tendon is united to the pericardium. This part, during expiration, is the highest part of the middle portion of the diaphragm. In expiration, the lateral muscular zone of the diaphragm lies in contact with the lower part of the thoracic wall (Fig. 465). During inspiration, the muscle fibres contract and draw the central tendon downwards, so that the lower surface of the lungs descends. The enlargement of the lungs at the lower part of the thorax is aided by the abduction of the floating ribs, produced by the contraction of the *quadratus lumborum* and *deep costal* muscles. In this contraction the diaphragm presses on the contents of the abdomen, so that the abdomen swells out with each inspiratory movement. The middle of the central tendon, where the heart lies, moves less than the two domes, and the part where the vena cava passes through the tendon is practically stationary during normal respiration. In deep inspiration, however, both this part as well as the rest of the pericardial attachment is forcibly depressed towards the abdomen. In quiet breathing, when observed by the X-rays, the mean descent of the right dome in inspiration has been found to be about 12·5 mm., and of the left dome 12 mm. We may say, roughly, that the average descent of the diaphragm during normal respiration is about half an inch. The viscera and the intra-abdominal pressure play an important part in determining the movement of the diaphragm, and especially in preserving the abduction of the lower ribs and so furnishing a fixed point for the muscular fibres of the diaphragm. If the contents of the abdomen are removed from a living animal, the ribs are drawn inwards every time the diaphragm contracts. In children with weak chest walls, and with respiratory obstruction, we may often see a depression round the lower part of the chest, which corresponds to the line at which the diaphragm leaves the chest wall, so that the distending force of the abdominal pressure on the bony walls of the thorax abruptly gives place to the pull of the distended lung. The contraction of the diaphragm may be regarded as a rhythmic fluctuation of tonus; in quiet breathing, at all events, only some of the fibres, and not always the same ones, "contract," which explains the absence of fatigue.

Rib Movements. The enlargement in the other diameters is effected by an elevation of the ribs (Figs. 465 and 466). Each pair of corresponding ribs forms a ring directed obliquely from behind downwards and forwards. With each inspiratory movement the ribs are raised and the horizontal distance between sternum and spinal column is therefore increased. Moreover, the ribs from the first to the seventh increase in length from above downwards, so that when they are raised, the sixth rib, for instance, occupies the situation previously taken by the fifth, and the transverse diameters of the thorax at this level become greater. With each inspiration, there is also a rotation of the ribs. In the expiratory condition, they are so situated that their outer surfaces are directed not only outwards but also downwards. As they are raised by the inspiratory movements, they rotate on an axis directed through the fore and hind ends of the rib, so that their outer surfaces are turned directly outwards. In this way a certain enlargement of the thoracic cavity is produced. As the thorax is raised, there is always some twisting of the rib cartilages.

The chief muscles raising the ribs in inspiration are the external intercostals, which act as explained later. The serrati postici and scaleni also contribute. It is probable that an important part is played by the extension

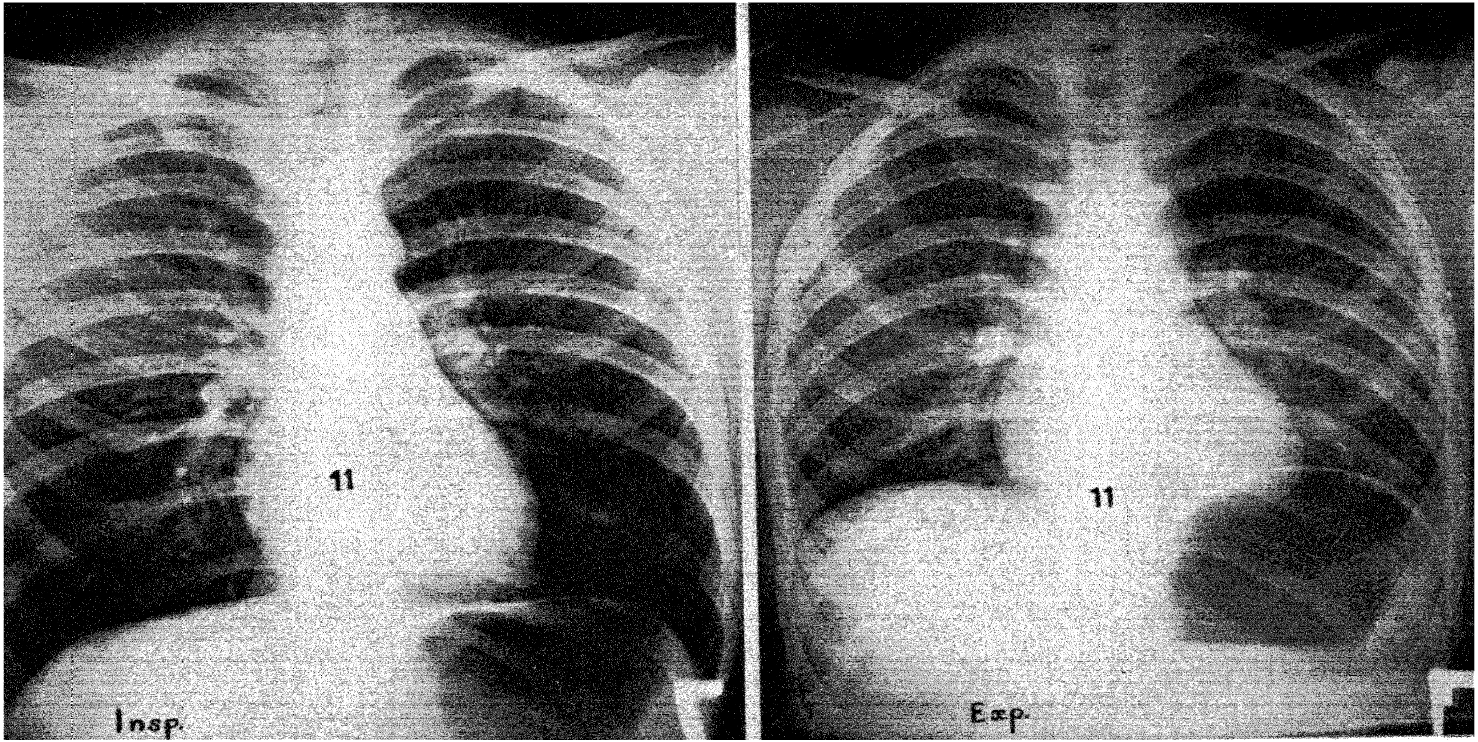

FIG. 465. Postero-anterior radiographs (negatives) of the thorax of a youth aged 18, taken at 6 feet distance. The level of the 11th thoracic vertebra is indicated as a landmark. In full inspiration note the darkness (*i.e.* transparency) of the lower half of the lung-fields, the width of the intercostal spaces and the outline of the heart, diaphragm, root of lung, and the gas-filled fundus of the stomach. In full expiration note the lessened opacity of lower half of the lung-fields, the obliquity of the ribs, the altered position of the heart, root of lungs and diaphragm, and the expansion of the gas in the stomach fundus. (Radiograph specially made by DR JAMES F. BRAILSFORD.)

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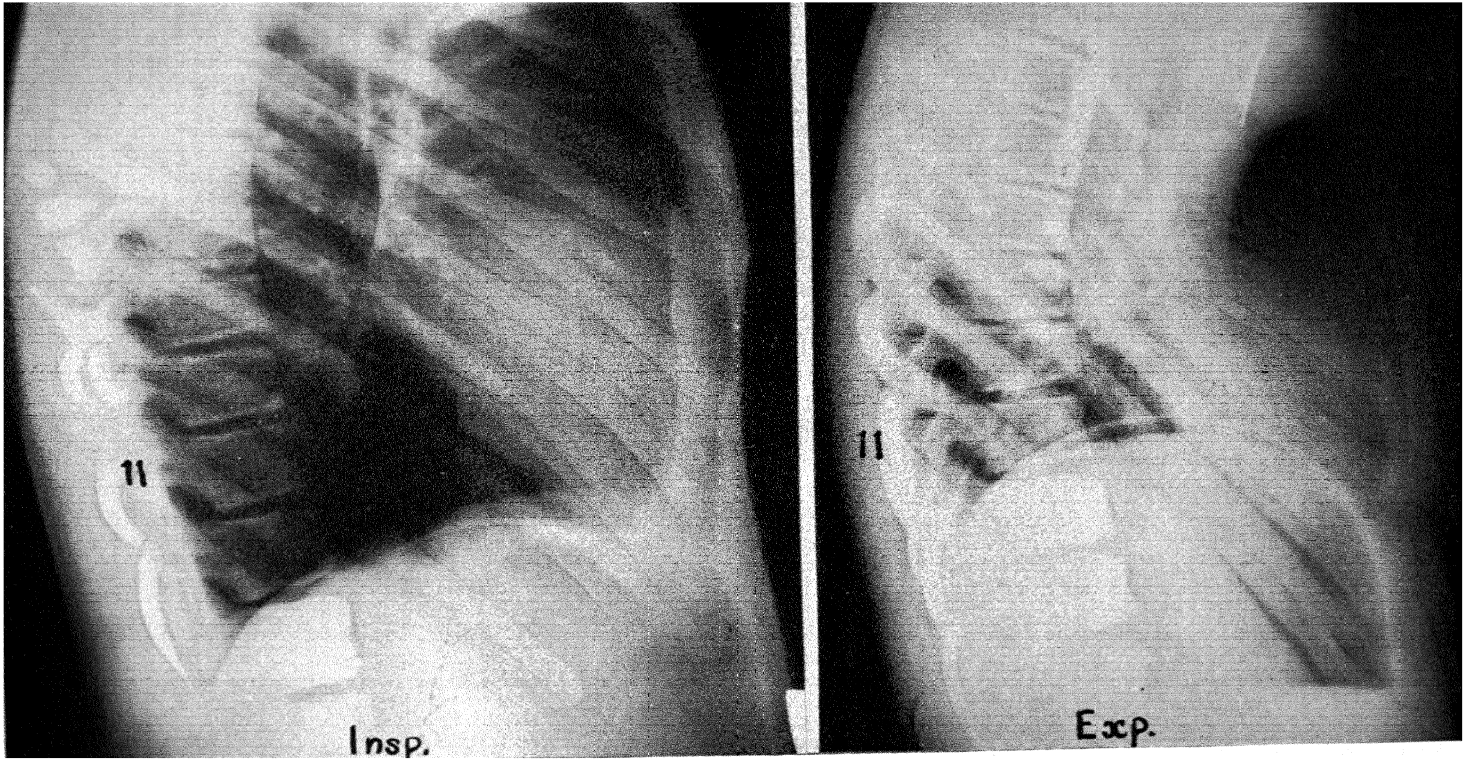

FIG. 466. Lateral radiograph of the chest of the same subject as the preceding figure. Note particularly the reduced obliquity of the ribs and the darkness of the lower lung-fields in full inspiration. (Radiograph specially made by DR. JAMES F. BRAILSFORD.)

[To face p. 745.

of the spinal column. This movement, which is specially marked at the upper part of the thorax, causes an increase in all three diameters of this cavity.

The muscles mentioned are the only ones normally engaged in carrying out inspiration. The *levatores costarum* are concerned, not in respiration, but in lateral movements of the spine. When, from any cause, the inspiratory efforts become more forcible, a large number of accessory muscles are brought into play. These are: the scaleni, sterno-mastoid, trapezius, pectoral muscles, rhomboids, and the serratus anticus.

The force exerted by the inspiratory muscles is nearly all spent in overcoming the elastic resistance of the lungs and costal cartilages. A free access of air is provided for by contractions of certain accessory muscles of respiration. When the glottis is observed by means of the laryngoscope, rhythmical separation and approximation of the vocal cords is observed, synchronous respectively with inspiration and expiration (Fig. 300, p. 485). When inspiration is laboured, the alæ nasi are dilated by the action of the dilator nasi. This movement of the nostril, which is constant in many animals, becomes very marked in children suffering from certain types of respiratory trouble.

Expiration. In expiration, the processes are reversed, and the cavity of the thorax is diminished in all three dimensions. Normal expiration is chiefly effected passively. When the inspiratory muscles cease to contract, the lungs, which were stretched by the previous inspiration, contract by virtue of the elastic tissue they contain, and the thorax itself sinks by its own weight, and by the elastic reaction of the stretched costal cartilages.

It must be remembered, however, that in a position of rest, the elasticity of the thorax is opposed to the elasticity of the lungs. Elasticity of the chest wall would, therefore, tend to produce inspiration. This factor would make inspiration easier at its onset, and would also present an impediment to the carrying out of expiration, so that towards the end of this act there is need for the active co-operation of muscular contractions. It seems possible that more or less muscular activity of the expiratory muscles is alternated with that of the inspiratory muscles. In fact, Sherrington's results on the co-ordination of muscular movements would lead us to assume inhibition of the tone, *e.g.* of the abdominal muscles, during inspiration, and active augmentation of their tone during expiration. Where the tone of the muscle is entirely lost, *e.g.* in the condition of visceroptosis, it has been observed that the diaphragm is thrown out of action, breathing being carried out chiefly by an elevation of the upper part of the thorax. Probably, under normal circumstances, the internal intercostal muscles also contract with each expiration. When the nerves to external and internal intercostal muscles are led off to recording oscillographs, showers of nerve impulses pass down at each respiration, to the external intercostals in inspiration and to the internal intercostals in expiration.* When the breathing is deepened the impulses are seen to be more frequent.

In forced expiration, muscles may take part—such as the serratus posticus inferior and the muscles forming the wall of the abdomen, *i.e.* the rectus, obliquus, and transversus abdominis muscles.

Although the action of the intercostal muscles has been a subject of debate and cannot even yet be regarded as definitely settled, physiological experiments serve on the whole to confirm the view first put forward by Hamberger, and based on a consideration of the direction of the fibres. The external intercostals pass from one rib to the next below, downwards and forwards. Hence, if a pair of ribs be isolated from the rest

* BRONK and FERGUSON. *Amer. Journ. Physiol.*, 1935, **110**.

of the chest wall, leaving the vertebral and costal attachments intact, contraction of these muscles will cause both ribs to rise. This result will be evident from a consideration of Fig. 467, where *ab* is a fibre of the external intercostal muscles passing from the rib *vs* to be attached to the rib *v's'* at *b*. When *ab* contracts, the tension it exerts on its two attachments can be resolved into two components *ac* acting downwards and *bd* acting upwards. *bd*, however, acts at the end of the long lever *bv'*, whereas *ac* acts at the end of the short lever *av*. Hence the raising effect will overcome the depressing effect, and both ribs will rise.

The fibres of the internal intercostals run in the opposite direction to the external

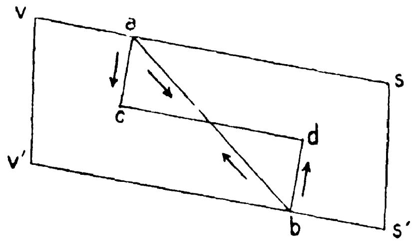

FIG. 467.

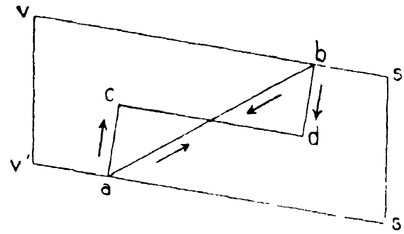

FIG. 468.

muscles, and from a consideration of Fig. 468 it is evident that their effect will be to depress any pair of ribs, thus acting as expiratory muscles.

Owing to the fact that the costal cartilages make an angle with the bony ribs, the fibres of prolongation of the internal intercostals, *musculi intercartilaginei*, have the same relation to their attachments that the external intercostals have to the bony ribs. Their action, therefore, must be to raise the cartilages and flatten out the angle between the cartilaginous and bony ribs, so that they must act with the external intercostals as inspiratory muscles.

Lung Movements. As the lungs are inflated their position changes

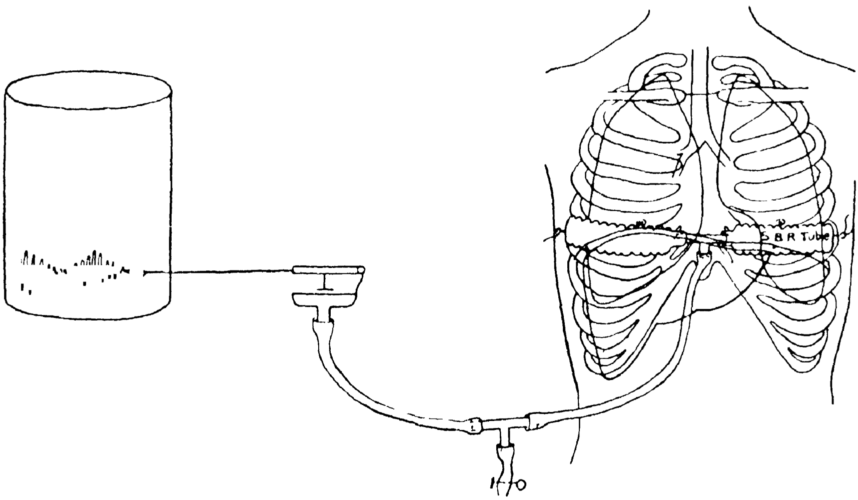

FIG 469. A convenient form of stethograph. The portion placed round the chest consists of corrugated (" Gorman ") tubing, which opens out like a concertina on inspiration. (HARRIS'S " Experimental Physiology.")

in relation to the thoracic wall. All parts are not equally distensible in the normal position of the lungs. There are three areas which are in contact with the nearly stationary parts of the thoracic wall and cannot therefore be directly expanded. These are (1) the mediastinal surface in contact with the pericardium and structures of the mediastinum; (2) the dorsal surface in contact with the spinal column and with the spinal segments of the ribs; (3) the apical surface lying in contact with the deep cervical

fascia at the root of the neck. The expansion of these portions of the lung is in a downward direction and is indirectly caused by the movement of the diaphragm. The roots of the lungs move, with inspiration, slightly forwards and downwards. With inspiration there is also an increase in length and width of all the air-tubes, even of the trachea and large bronchi, but especially of the smaller ones. The actual alveoli are probably little, if at all, stretched at inspiration, but merely become flatter and wider. At expiration, all these changes are reversed.

METHODS FOR STUDY OF RESPIRATORY MOVEMENTS. In man, the enlargement of the chest and the accompanying movements of the diaphragm, ribs, heart, root of lungs, &c can partly be seen on visual, or better on X-ray examination; records of the expansion of the chest or abdomen can be obtained by the use of stethographs, of which there are many patterns, mostly on the air-transmission principle (Fig. 469).

A method giving quantitative records is to place the man, or animal, in an airtight box, from which the head protrudes, the neck passing through an airtight collar. the interior of the box is connected to a volume recorder.* In animals a record may be made of movements of portions of the diaphragm, of the sternum or ribs, or of the larynx. A convenient method is to insert a small rubber balloon filled with air or water beneath the diaphragm and connect it with a water manometer or volume recorder Yet another method is to record the intrapleural pressure changes,† or the pressure changes in the trachea. The volumes of air breathed in or out may be measured by a gas-meter or spirometer through which the man or animal breathes by means of appropriately arranged valves. For details, practical books must be consulted ‡

The extent and boundaries of the lungs can easily be ascertained in the living subject by means of *percussion*. On tapping the finger laid on the chest, a sound is emitted, which varies with the nature of the subjacent tissues. If this is lung tissue filled with air, a clear resonant tone is obtained; where it is solid tissue, such as the heart, or a lung consolidated with inflammatory products, or the liver, a dull sound is obtained. It is easy to show that the resonant area of the chest increases with each inspiration. The apices of the lungs extend about one inch above the clavicle anteriorly, and reach as high as the seventh spinous process behind. During moderate expiration the lower margin of the lungs extends, in front, from the upper border of the sixth rib at its insertion to the sternum, and runs obliquely downwards to the level of the tenth rib at the back of the chest. During the deepest inspiration the lungs descend, in front, to the seventh intercostal space, and behind, to the eleventh rib, while during deepest possible expiration, the lower margins of the lungs are elevated almost as much as they descend during inspiration. In the front of the chest a triangular space can always be marked out over the heart, where the note obtained on percussion is dull. This space is bounded on the right by the left border of the sternum, and extends out as far as the cardiac apex, being bounded above by the fourth costo-sternal articulation and below by the sixth costal cartilage.

BREATH SOUNDS. If the ear be applied to the chest wall, either directly or through the medium of a stethoscope, each inspiration is found to be accompanied by a fine rustling sound, the 'vesicular murmur.' It is thought to be caused by the sudden dilatation of the air vesicles during inspiration, or perhaps by the current of air passing from the terminal bronchioles into the wider respiratory bronchioles. It is important to remember that this sound is heard only during inspiration, and over healthy lungs. On listening over the larger air passages, *i.e.* the larynx, trachea, and bronchi, we hear a much louder sound which accompanies both expiration and inspiration, and may be compared to a sharp whispered *hah*. This is known as the 'bronchial murmur.' It can be heard also at the back of the chest between the scapulæ at the level of the fourth dorsal vertebra, where the trachea bifurcates. In all other parts of the chest the healthy lung prevents the propagation of this sound to the chest wall. If, however, the lung is solid, as occurs in pneumonia, it conducts the sound easily from the large air tubes to the chest wall. Bronchial breathing at any part of the chest, other than that immediately over the air tubes, is therefore a sign of consolidation or cavitation of the lung. Absence of breath sounds at any part of the chest implies, either that air is not entering that part of the lung, or that the lung is separated from the chest wall, *e.g.* by effused fluid.

* HALDANE and PRIESTLEY. *Journ. Physiol*, 1904, **32**, 242.

† FRANKLIN and GILDING. *Journ. Physiol*, 1932, **75**, Proc i.

‡ DOUGLAS and PRIESTLEY. "Human Physiology." Oxford, 1924. LAMB. "An Introduction to Human Experimental Physiology." London, 1930.

INTRATHORACIC AND INTRA-ABDOMINAL PRESSURES. Even at the end of normal expiration the healthy adult lungs are in a stretched condition. This is shown by the fact that if an opening be made into the pleural cavity, air rushes into the opening (*pneumothorax*) * and the lungs collapse, driving a certain amount of air out through the trachea. Since the lungs are always tending to collapse, it is evident that they must exert a pull on the thoracic wall. The pressure in the pleural cavity, between the two layers of the pleura, is called the "intra-pleural pressure." Owing to the pull of the lungs it is below the pressure of the surrounding atmosphere and is sometimes, though inaccurately, called a "negative pressure" in consequence. It is often imagined, though quite wrongly, that the existence of this "negative pressure" is in some way essential to the performance of inspiration, in which act it becomes still more "negative." Actually, of course, the pleura covering the surface of the lungs is in contact, except for a film of pleural fluid, with the pleural layer lining the chest wall, so that the "pleural cavity," in the normal chest, is almost non-existent, and in any case does not contain air. The so-called "negative pressure" is again often, and once more wrongly, stated to be what keeps the two layers of the pleura in contact. So far from being the cause, it is, in fact, an effect, firstly of this contact, secondly of the normally stretched condition of the lungs. The effect of the contact between the two pleural surfaces is that, in order to separate them, the film of liquid by which they are wetted must be broken. To do this requires considerable force, a film of water at body temperature being able to sustain a tension of 3,600 mm. Hg./sq. cm. without rupture.† Everyone knows that what makes a dry rubber sucker adhere is not suction within but pressure of air from without; remove this air pressure and the sucker fails to stick. But a wet sucker would still adhere unless pulled sufficiently to break the fluid film. The lungs, therefore, cling to the chest wall because they fill all the available space of the thorax. If, in so doing, they are stretched, there will be a tendency, proportional to the stretch, for the lungs to collapse, and this will cause the so-called negative pressure. If the atmospheric pressure inside the lung $= P$, and the elastic recoil of the lungs $= p$, then the two layers of pleura will be held together by the force $P - p + S$, the "negative pressure" of course also being $= p$, and exerted on the whole contents of the thorax, and S being the initial force required to break the liquid film. In the expiratory position the intrapleural pressure is 3 to 5 mm. Hg. below the atmospheric; in normal inspiration the difference is about 5 to 10 mm. below atmospheric. If the lungs are fully distended by a deep inspiration the elastic forces are brought more into play, and the negative pressure in the pleura may amount to 30 mm. But it must be clearly understood that if the lung tissue had no elastic recoil, so that there was no 'negative pressure,' the act of inspiration would not be affected in any way. But respiration becomes impossible directly free openings are made into the pleural cavities on both sides of the chest. With each inspiratory movement, air rushes in through these openings, so that the thoracic movements can no longer exert any influence on the volume of the lungs.

The negative pressure in the thorax is diminished by any factor decreasing the elasticity of the lung tissue. Thus, in an old man, where the elastic tissue is degenerated and the alveoli are enlarged, giving rise to the condition known as *emphysema*, the lungs may collapse only slightly, or not at all, on

* GRAHAM. Alterations of Intrapleural Pressure and their Significance. HARVEY LECTURES, XIX, 1924, 123. LIPPINCOTT.

† BURNS. *J. Physiol.*, 1940, **98**, 26P.

opening the chest. The lungs do not collapse on making an opening in the chest of a new-born mammal; but this is owing to the fact that they completely fill the thorax in the expiratory position, and it is only later that, with the growth of the ribs, the thorax gets, so to speak, too large for the lungs, which are therefore stretched to fill it.

The pressure in the abdominal cavity is of course raised when the diaphragm descends during inspiration, so that the pressure fluctuations in the thoracic and abdominal cavities are reciprocal in direction (Fig. 470). Whether the actual pressure at any point in the abdominal cavity is above or below that of the outside air will obviously depend greatly upon the position considered, the posture of the body, the tension on the abdominal walls, &c., but in any case, with normal breathing, the pressure in the abdomen will rise, as that in the thorax falls, during inspiration, and *vice versâ*. This is a fact of the greatest importance in several directions.

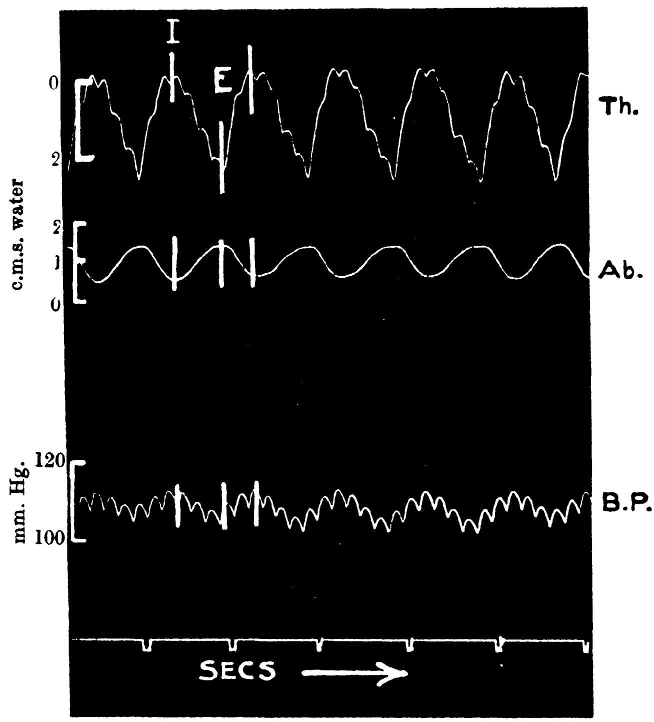

FIG. 470. Fluctuations of intrathoracic (Th) and intra-abdominal (Ab.) pressure (in terms of cm. water) and of arterial blood pressure (in mm. Hg) of an anæsthetised cat breathing quietly. I = inspiration begins. E = expiration begins. (H. P. GILDING.)

If a manometer be connected with one of the nostrils, so as to register the pressure in the air cavities, it is found that there is a negative pressure of — 1 mm. Hg with inspiration, and a positive pressure of 2 or 3 mm. with expiration. With forced inspiration, the pressure may amount to — 57 mm. Hg, and with forced expiration there may be a pressure of + 87 mm., the latter mainly due to action of the abdominal muscles.

PULMONARY VENTILATION. Under no circumstances can we expiration quite empty the lungs of air. At the end of the most forcible

expiration the alveoli and all the air tubes still contain air. When breathing quietly, a man takes in and gives out at each breath about 500 c.c. of air, measured dry and at 0° C. If measured moist and at the temperature of the body, viz. 37° C., the volume would be about 600 c.c. This amount is known as the *tidal air*. By means of a forcible inspiratory effort, it is possible to take in about 2000 c.c. more (*complemental air*). At the end of a normal expiration a forcible contraction of the expiratory muscles will drive out about 1,300 c.c. more (*reserve or supplemental air*). These three amounts together constitute

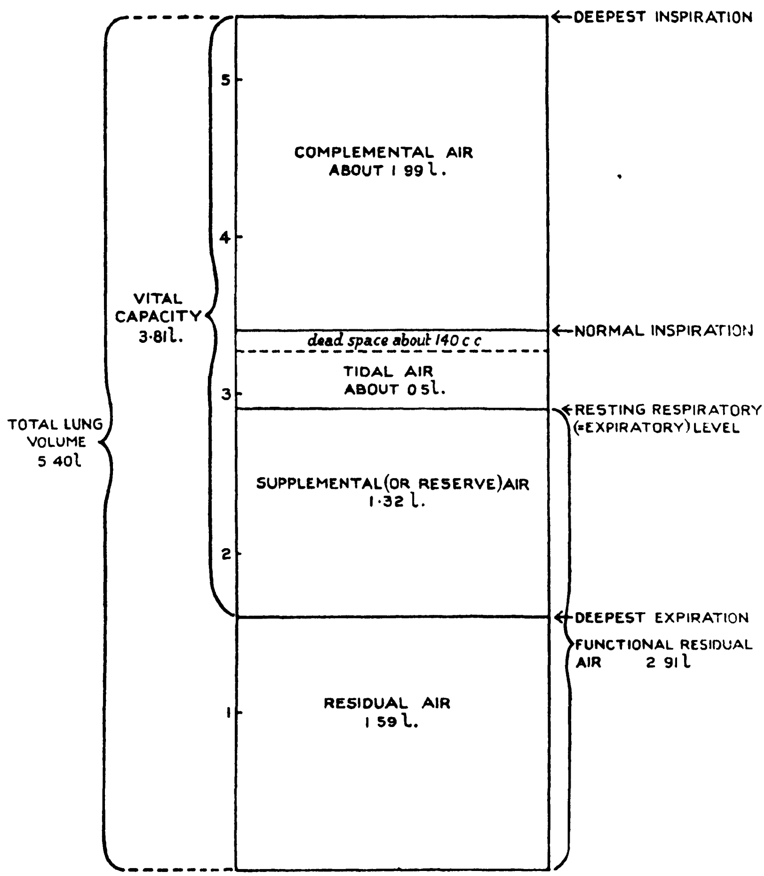

FIG. 471. Lung volumes. (Data from Aslett *et al.*)

the '*vital capacity*' of an individual. This total may be determined by means of the spirometer, a small gasometer with a gauge by which the amount of air in it can be at once read off. The person to be tested fills his lungs as full as possible, and then expires to the utmost into the spirometer. Individual variations in vital capacity are great (2·5—5·5 l.), and depend to a considerable extent on physical build Of the total lung volume, the vital capacity of British subjects averages about 71 per cent., supplemental 25 per cent., functional residual air 54 per cent., residual air 29 per cent.* The *functional residual air* is supplemental plus residual air (Fig. 471). The vital capacity is greatest when standing, and least when lying in the prone position,

* ASLETT, D'ARCY, HART and MCMICHAEL. *Proc. R. S.*, 1939, **126B**, 502.

the difference being about 300 to 500 c.c. The loss of vital capacity when lying down is due to upward displacement of the diaphragm by the abdominal viscera, and also, according to Hamilton and Morgan,* to the fact that blood accumulates in the lungs. In cardiac disorders with weakness of the left ventricle, patients often are unable to lie down because of the pulmonary congestion and breathlessness which ensue (orthopnœa).

Increased depth of breathing is called *hyperpnœa.* When for any reason the pulmonary ventilation approximates to the volume of the vital capacity, the breathing becomes laboured, and is called *dyspnœa.* Voluntary hyperpnœa is called *forced breathing.*

The air left in the lungs after the most vigorous expiration is known as the *residual air.* As a result of actual determinations, we may assume the

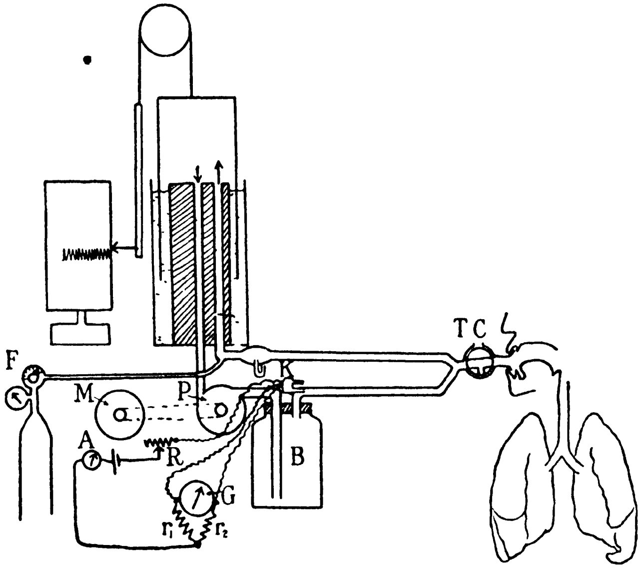

FIG. 472 Spirometer arranged to determine lung volume P, the circulating pump; B, bottle with absorbent for CO_2; volume kept constant by running in O_2 from F at same rate as usage; K, a katharometer connected to ammeter A and galvanometer G, for ensuring constant composition of mixture (HERRALD and McMICHAEL, *Proc. R. S.*, 1939, **126B**, 491.)

residual air in the lungs as something between 1000 and 1700 c.c., generally about 1600 c.c. It is not reduced in the recumbent posture.

The various lung volumes can be accurately determined by the use of a recording spirometer as follows: †

(1) The subject is connected to the spirometer arranged as in Fig. 472 and a record of the rate of oxygen consumption is taken in the usual way. During this time records are also made of *vital capacity, complemental air* and *supplemental air.* The subject is now disconnected.

(2) It now remains to determine *functional residual air.* The principle of the method is to let the patient breathe from an atmosphere of *known volume* and of *known composition,* but differing from that of the air by being greatly enriched with oxygen. The composition of the mixture is kept constant by adding oxygen at the same rate as the subject uses it. The CO_2 is absorbed. In seven minutes equilibrium is established, the patient is disconnected and the mixture analysed for O_2 and N_2.

* HAMILTON and MORGAN. *Amer. J. Physiol.*, 1932, **99**, 526.

† HERRALD and McMICHAEL, *Proc. R. S.*, 1939, **126B**, 491.

A calculation shows the functional residual air. Thus, suppose the patient's lungs at the start contained x l. of air which = 0·791 x l. of N_2, and suppose the spirometer contains to begin with 2 l. of air (= 2 × 0·791 l. N) plus 3 l. of O_2. The volume is the same at the end because oxygen is added as used, and all that has happened is that the lung gases and spirometer gases have been mixed together. Suppose there is 50 per cent. of N_2 in the final mixture ; the functional residual air is x l.

Then we have

$$2 \times 0{\cdot}791 + 0{\cdot}791\,x = 0{\cdot}50\,(x + 5)$$
$$0{\cdot}291\,x = 0{\cdot}918$$
$$\therefore \quad x = 3{\cdot}16 \text{ l.}$$

(3) If the supplemental air was found to be, say, 1·56 l., the *residual air* would be 3·16 − 1·56 l. = 1·6 l.

Of the 500 c.c. of tidal air taken in at each inspiration, only a certain part reaches the alveoli, part being required to fill the air tubes, trachea, bronchi, and bronchioles which lead to the air cells. The volume of these air tubes is called the '*dead space,*' and is normally taken as about 140 c.c., so that, of the 500 c.c., about 360 c.c. actually reach the alveoli. For the same reason, the expired air represents the air from the alveoli (360 c.c.), diluted with 140 c.c. of dead space air which has remained in the air tubes and undergone very little change, other than the elevation of temperature and saturation with aqueous vapour. There is no doubt, however, that it is incorrect to regard the volume of the dead space as constant, since not only do the branches of the bronchial tree show at inspiration an increase, and at expiration a decrease, in all dimensions, but the volume of the physiological dead space no doubt varies with alterations in the state of the bronchial musculature and various other factors. It is, for instance, increased in exercise.*

The velocity with which the air enters and leaves the respiratory tract may be investigated by means of an instrument on the principle of the Poiseuille tubes, and called a *pneumotachograph*.† It shows that the maximum speed of movement of the air is at the commencement of inspiration and expiration, and the minimum speed just before the end of each phase.

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ARTIFICIAL RESPIRATION. Under various conditions the respiratory movements may cease, and it is then necessary, in order to support life, to cause air to enter the lungs by some artificial means. This may be accomplished in various ways by which air is forced or drawn into the lungs, and forced or drawn out of them, at suitable intervals. The most easily controllable method of doing either is by means of suitable air-pumps, but when these cannot be used, quite efficient ventilation can be obtained by simple means, as described below.

Inflation Methods. These methods are most commonly applied to anæsthetised animals. In the simplest method, a suitable tracheal cannula is tied into the trachea, and warm moist air is delivered to the tube rhythmically from a pump. The tracheal cannula has a side tube which can be partially closed by a screw clamp ; as each blast of air is delivered, some excess escapes by the side tube, and the rest of it inflates the lung ; between the blasts the lungs collapse and the air expelled escapes by the side tube. When ventilation is being carried out with gases other than atmospheric air, this method

* DOUGLAS and HALDANE. *J. Physiol.*, 1912, **45**, 235.

† FLEISCH. *Hdbch. d. biolog. Arbeitsmethoden.* (Ed. Abderhalden.) *V.* **8**, 845.

is wasteful. In such cases it is better to use a more elaborate pump, such as the one designed by Starling ("Ideal" pump). In this the side tube is closed by a mechanical device while the pump is delivering, so that the whole of the air delivered is kept in the closed system of lungs plus tubes; at the end of the stroke the side tube is opened and the lungs collapse. In a third type of pump, the trachea is connected to a double pump which alternately forces air into the trachea from one barrel and draws it out again into the other one. The last two methods both allow of respiration being carried out with any desired gas mixture. With suitable modifications in the way of masks instead of tracheal cannulæ, similar devices have been applied for use on the human subject. It has been found by Yandell Henderson, Drinker and others that the administration, by some such method, of oxygen containing 7 per cent. carbon dioxide is most valuable in the resuscitation of persons apparently drowned or suffering from coal gas poisoning, and various apparatus for the purpose have been introduced.

Continuous Insufflation. If a flexible tube of small diameter be passed down the trachea as far as its bifurcation, it is possible to maintain life without any movement of the chest at all, by injecting a constant stream of oxygen, with or without 5 per cent. CO_2, by way of the tube, so that the lungs are slightly distended. This has enabled operations to be carried out on patients with opened thorax.

*Chamber Method.** In this method the subject is placed with his body in a chamber

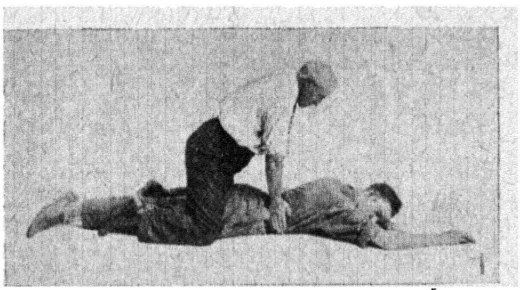

FIG. 473. Schafer's method of artificial respiration. (DRINKER.)

and the head outside, the neck passing through an airtight collar in the wall. The chamber is airtight, and when air is sucked out of it by a rhythmically operated pump, the subject's chest is expanded and air is drawn into his lungs; the reverse happens when air enters the chamber again.

The Bragg-Paul Pulsator† is a simple mechanical device which can be operated by a motor, or by water-pressure for indefinitely long periods.‡ It consists of a rubber air-bag wrapped round the chest and rhythmically inflated and deflated.

Emergency Methods. In animals rhythmic compression of the chest is often adequate. In man, the best method is that described by Sharpey-Schafer, § and generally adopted throughout England and America, for the resuscitation of asphyxiated persons. The subject is placed in the prone position, with the head slightly to one side; the mouth is cleared and the tongue pulled well forward. The operator, kneeling either at the side, or astride, of the subject, places the palms of his hands flat on the subject's trunk, just over or below the lowest ribs and then by leaning forward presses *gently* forwards and upwards so as to exert pressure there, for, say, two seconds (Fig. 473). He then leans back a little to release the pressure for two seconds, and then again repeats the forward movement, and so on. In this way air is forced out of the chest when pressure is applied, because the diaphragm is pushed up; when the pressure is released, the elastic recoil

* DRINKER and MCKHANN. *J. Amer. Med. Assoc.*, 1929, **92**, 1658.
† PAUL. *Proc. Roy. Soc. Med.*, 1935, **28**, 38.
‡ TOOKEY KERRIDGE. *Lancet*, 1934, i., 786.
§ SCHAFER *Trans. Med. Clin. Soc. Lond.*, 1904, **87**, 609, *J. Amer. Med. Assoc.*, 1908, 51, 801

draws air into the chest. It is easy by this method, *with which every person of adult age should be familiar*, to maintain normal ventilation in an unconscious subject for long periods without undue fatigue on the part of the operator.

With patients under an anæsthetic for surgical operation, and lying in the supine position, this method cannot readily be used ; it should be known, therefore, that quite good results can be obtained by similarly applying intermittent pressure on the epigastrium, by which means also the diaphragm is made to ascend. *It cannot be too strongly insisted that pressure on the upper ribs is almost useless.* In both of these methods it is advantageous, where possible, to let the subject inhale, from a suitable mask, a mixture of oxygen and 7 per cent. carbon dioxide.

Another simple method which can be carried out for long periods is the rocking method of Eve.* The patient is placed in the prone position on a stretcher, which is rocked on a pivot like a see-saw 45 degrees up and down 12 to 15 times a minute. When the head is downwards the weight of the abdominal viscera pushes the diaphragm up, and in the feet-down position the diaphragm descends. The tidal air by this method is slightly greater than in Schafer's method,† viz. 450 to 600 c.c./min.

THE BRONCHIAL MUSCULATURE

All the air tubes have in their walls a middle layer of plain muscle. When the lung is normally distended, this muscle is neither circular nor longitudinal, but forms an oblique network, which by its constriction narrows the lumen and decreases the length of the tube at the same time. The muscle only appears circular and compact when microscopically examined in the collapsed state (Macklin). The guinea-pig is perhaps the only animal in which the bronchial muscle is definitely circular and also compact,‡ and in this animal the thickest muscle is in the secondary *bronchi* (just after they arise from the primary) ; this is the site of occlusion which causes death from asphyxia in anaphylaxis in the guinea-pig. Immediately internal to the muscle coat lies a prominent layer of longitudinally coursing branched elastic fibres, and from this elastic lamina fibres extend amongst the muscle fibres and form, as in the arteries, gut, spleen, &c., an intimate ' tissue partnership.' In expiration, contraction of this ' myo-elastic ' system plays an important part in decreasing the size of the whole lung, and in addition decreases the capacity of the dead space by shortening and narrowing the airway. Contraction of the bronchial muscle has the following effects : (1) a constriction of the bronchi and bronchioles ; (2) a diminution of the air space of the lung, and therefore of the volume of the lung ; (3) an increased resistance to the passage of the air into, and out of, the alveoli.

Changes in the condition of contraction of the bronchial muscle may be studied in various ways. In one method, artificial respiration is carried out, a constant volume of air being blown in and sucked out at each respiration, while alterations in pressure due to the varying resistance of the bronchioles may be recorded from a tracheal side tube (Fig. 474, T.P.). Any diminution in the calibre of the bronchioles must increase the resistance to the incoming current of air, and so cause a rise of pressure in the tracheal tube. By a second method, artificial respiration at a constant pressure is made use of, and alterations in lung volume at each pump stroke may be recorded by

* Eve. *Lancet*, 1932, *ii.*, 995.
† Killick and Eve. *Lancet*, 1933, *ii.*, 740.
‡ Miller. *Amer. Rev. Tub.*, 1921, 5, 689.

enclosing the lung in a plethysmograph * (Fig. 474). In a third method, the lungs may be excised and the air tubes perfused from the trachea with a suitable warm saline solution, while the pulmonary circulation is also similarly perfused. The bronchial perfusion fluid flows away through scarifications made on the lung surface, while the resistance is measured by a tracheal side tube and suitable manometer.† The action of drugs or inorganic ions may conveniently be studied by this method. By all these methods it has been shown that stimulation of the peripheral end of the vagus or presence of acetyl choline causes constriction of the bronchioles (Fig. 474), while Dixon and Ransom ‡ (Fig. 475) have by the second method shown that inhibitor fibres supply the bronchial muscle from the sympathetic nervous system. The action of adrenaline also causes dilatation of the bronchi. Histamine causes

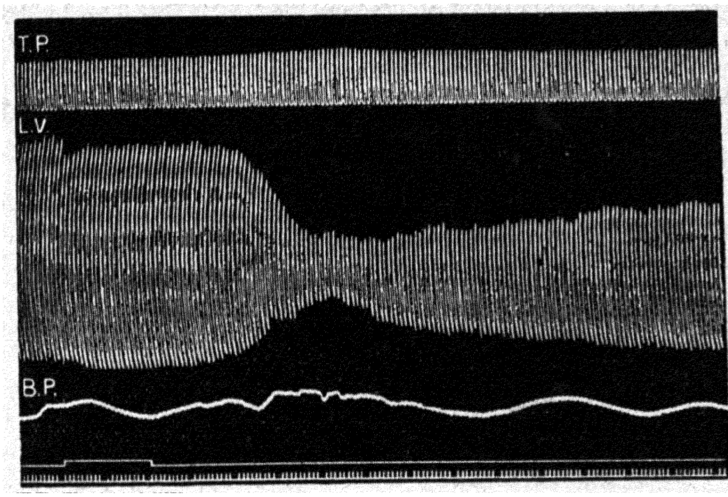

FIG. 474. Tracings of the Volume Changes of the Lung, with constant Variations of Tracheal Pressure. (DIXON and BRODIE.)

T.P. tracheal pressure. L.V. lung volume. B P. blood pressure (Zero B.P. 17 m.m. below time marker). Showing constriction of bronchial musculature as a result of vagus excitation.

intense constriction of bronchi through its direct action on the muscle,§ and this, like all reactions of plain (and other) muscle, is diminished by reduction of the calcium content of the fluid in which it is bathed.||

In man the bronchi have been studied with the aid of the bronchoscope,¶ and by X-rays after the injection of radio-opaque substances ('lipiodol') into the bronchial tree.** By both these methods the bronchi have been shown to have a rhythm synchronous with and similar to that of the vocal cords (p. 485), dilating in inspiration and constricting in expiration. The cause of this rhythm is not known. In part it is no doubt passive, and represents a general alternate expansion and contraction of the whole of the lung in consequence of the respiratory movements. But many believe it to be also, in part, active. In so far as this is the case it may be regarded as a part of the normal respiratory movement, caused by the discharge of nervous impulses from the respiratory centre; we may suppose that, at each inspiration, the broncho-constrictor

* DIXON and BRODIE. *J. Physiol.*, 1903, **29**, 97.
† McDOWALL and THORNTON. *J. Physiol.*, 1930, **70**, Proc. xliv.
‡ DIXON and RANSOM. *J. Physiol.*, 1913, **45**, 413.
§ THORNTON. *Quart. J. Exp. Physiol.*, 1932, **21**, 305.
|| GILLESPIE and THORNTON. *J. Pharmacol.*, 1932, **45**, 419.
¶ JACKSON. Bronchoscopy and Œsophagoscopy. 2nd edition, 1927. (Saunders.)
** REINBERG. *Brit. J. Radiol.*, 1925, **30**, 451; BULLOWA and GOTTLEIB. *Am. J. Med. Sci.*, 1920, **160**, 98

impulses passing down the vagi are inhibited and that broncho-dilator impulses are sent down the sympathetic fibres to the lungs ; at expiration the reverse happens.

Peristaltic movements have also been described in the bronchi and are markedly brought into play on the introduction of foreign bodies (Reinberg). (Spontaneous rhythmic waves have been observed in the bronchi of the chick embryo by LEWIS.*) These represent a protective mechanism of the lung, which thus raises unwelcome fluid

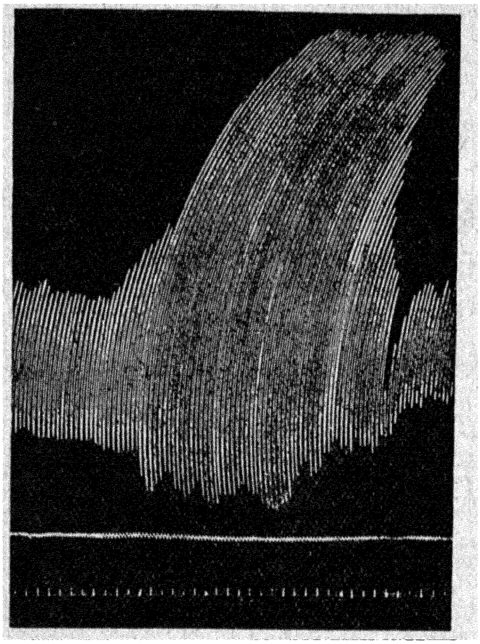

FIG. 475. Effect on Lung Volume of stimulation of post-ganglionic fibres from the ganglion stellatum. Time = 5 secs. (DIXON and RANSOM, *J. Physiol.*, 1912, **45**, 413.)

to those parts of the airway (primary and secondary bronchi) where the cough reflex can be originated, by which it is finally expelled.

In asthma, the excessive tonus of the bronchial muscle will cause obstruction to the passage of air in and out of the lung, expiration becoming more difficult than inspiration, probably owing to the rhythm mentioned above. Whether this hypertonous depends on chemical factors or not is at present unknown, but it is clear that nervous elements can be superimposed to add to the difficulties of the already complicated clinical picture in this condition.

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THE CHEMISTRY OF RESPIRATION

EXPIRED AND ALVEOLAR AIR

Expired Air. The tissues of a man of 70 kg. body weight consume, on an average during working hours, about 400 c.c. oxygen per minute. This oxygen is used for the oxidation of various materials in the body, of which the element carbon is a principal constituent. The tissues, in consequence, produce large quantities of carbon dioxide. The oxygen required by the

* LEWIS. *Am. J. Physiol.*, 1924, **68**, 385.

tissues is taken from the blood, and the carbon dioxide formed is, in return, passed out into the blood. Hence, the venous blood contains less oxygen than arterial blood, but it contains more carbon dioxide. In its passage through the lungs, the blood is again arterialised, oxygen passing into it and CO_2 out, owing to an exchange with the air in the alveoli. This air is continually renewed by breathing, and we therefore find that the expired air contains less oxygen, and more carbon dioxide, than that which is inspired. Its composition is not constant, however.

Expired air can be collected for analysis or measurement by the use of the Douglas bag (Fig. 476). The subject breathes through the valves,

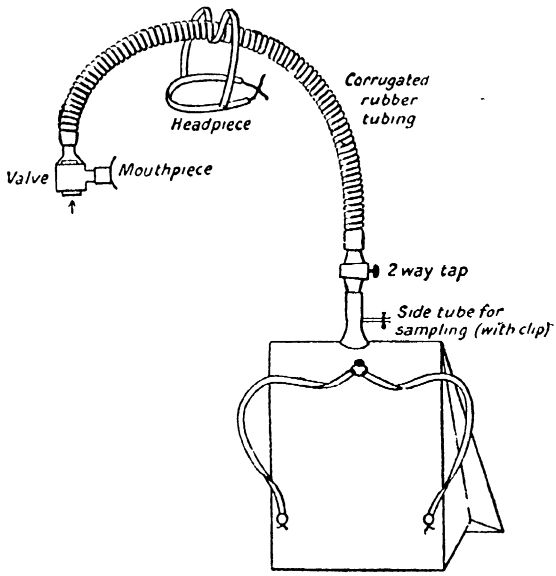

FIG. 476. Douglas' bag, with valves for collection of expired air.

which are so arranged that air from the outer atmosphere enters at inspiration, and is drawn into the lungs, while at expiration the expired air passes through the other valve, and into the bag, which is made of an impermeable fabric. Samples of the air can be drawn off from the bag for analysis, or the volume of expired air collected in a given time measured by expressing the contents of the bag through a gas meter.

The expired air of normal resting persons at sea-level contains about 3·0 to 4·5 per cent. of carbon dioxide, and 16·0 to 17·5 per cent. of oxygen. As a very rough average, it compares as follows with the inspired air :

	Inspired Air		Expired Air
Oxygen	20·95 vols. per cent.	...	16·4 vols. per cent.
Nitrogen (including argon)	79·01 „ „	...	79·5 „ „
Carbon dioxide . .	0·04 „ „	...	4·1 „ „

These figures refer to the dry gases, at N.T.P. Under normal circumstances, inspired air contains a variable amount of aqueous vapour and has a variable temperature. Expired air is nearly saturated with aqueous vapour and, in the trachea, has a temperature of about 37° C. The tension of aqueous vapour at this temperature is 47 mm. Hg. Thus when a man is breathing air at a pressure of 760 mm. Hg, the pressure of the mixture of

permanent gases in the alveoli of his lungs will be only 760 – 47, *i.e.* 713 mm. Hg.*

Calculation from these data shows that, whereas about 250 c.c. of oxygen are used up per minute by a normal resting man, only about 215 c.c. of carbon dioxide are added to the expired air in the same time, while the total *volume* of nitrogen leaving the lungs is the same as that taken in. In the analysis, the nitrogen *percentage* is seen to be higher in the expired air. This is because the volume of air expired is less than that inspired, owing to the disappearance of a certain amount of oxygen, without the production of a corresponding amount of carbon dioxide, so that the *relative* amount of nitrogen is slightly increased. The reason for this disappearance of oxygen is that when fats and proteins are being consumed, some of it is used for the combustion of hydrogen and for the partial oxidation of nitrogen, as well as in burning carbon. The ratio of the volumes $\frac{CO_2}{O_2}$ which are exchanged in a given time, and which is called the *respiratory quotient*, is normally about 0·85, but varies according to the nature of the materials subjected to combustion in the body and to other conditions.

Alveolar Air. As already explained, only about 360 c.c. of the 500 c.c. of air drawn in at an average breath reach the alveoli, the other 140 c.c. being required to fill the dead space. Hence, the alveolar air must contain more carbon dioxide and less oxygen than the mixed expired air, which consists of alveolar air *plus* the unchanged dead space air, which precedes it on expiration.

At the end of an expiration, the dead space has been swept out by, and remains filled with, alveolar air. Based on this fact, a sample of alveolar air may be obtained for analysis in the following way (Haldane and Priestley).† A piece of indiarubber tubing is taken, of about 1 inch diameter and 4 feet long. Into one end (Fig. 477) is fitted a mouthpiece, near to which is a gas sampling-tube, which is provided with three-way taps at the upper and lower ends. Before an experiment the sampling-tube is completely evacuated. The subject of the experiment, after breathing *normally* a few times, at the end of a normal inspiration puts his mouth to the tube, expires quickly and deeply, and closes the mouthpiece with his tongue. The tap of the sampling-tube is then turned, and the air near the mouthpiece, which is that last expelled from the lungs, rushes into it. The tap of the tube is then turned off, and the gas may be removed for analysis. A similar sample is then taken, in which the subject expires deeply at the end of a normal expiration. This sample will contain slightly more CO_2 and less O_2 than that obtained at the end of inspiration. The mean of the two samples is taken as the average composition of the subject's alveolar air.

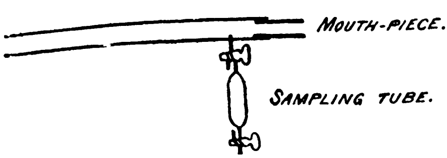

FIG. 477.

The composition of the alveolar air in resting subjects at normal atmospheric pressure is nearly constant, though showing variations from one individual to another. We may take the average composition for adult men at 760 mm. pressure, to be as follows :—

* This is on the assumption that the alveolar air is in equilibrium with *water* at 37° C. Direct experiment by CHRISTIE and LOOMIS (*J. Physiol.*, 1932, **77**, 35) gives a tension of aqueous vapour of only about 45 mm. Hg in quiet breathing, and still less during hyperpnœa.

† HALDANE and PRIESTLEY. *J. Physiol.*, 1904, **32**, 225.

MEAN COMPOSITION OF ALVEOLAR AIR. (Adult Men.)

	Volumes per cent. (dry).	Pressure mm. Hg. (moist.)
Carbon dioxide	5 50	39
Oxygen	13·80	103
Nitrogen	80·70	571
Water vapour	0 00	47
	100 00	760

The range for CO_2 pressures is about 32 to 45 mm. for men.

In children and women the pressure of carbon dioxide is on the average a little less, and the oxygen a little more.

The difference between the composition of expired air and alveolar air is determined by the dilution of the alveolar air with that contained in the dead space. Hence, with shallow breathing there will be a large difference, but this will decrease with increased depth of respiration. Thus, if the alveolar air contained 6 per cent. CO_2 and the dead space amounted to 150 c.c., the expired air would contain only 3 per cent. CO_2 when the person was taking in only 300 c.c. at each respiration. If, however, he was breathing slowly and deeply, so as to raise the tidal air to 1500 c.c., only one-tenth of this would be represented by the dead space, and the expired air would contain nine-tenths as much CO_2 as the alveolar air, *i.e.* 5·4 per cent.

THE DEAD SPACE can be determined by measuring the volume and estimating the composition of the air in an average expiration, and by finding the composition of the alveolar air. Then dead space

$$= \frac{\text{Tidal air vol.} \times (CO_2 \text{ per cent. in alveolar air} - CO_2 \text{ per cent. in expired air})}{CO_2 \text{ per cent. in alveolar air}}$$

The changes in the composition of alveolar air with each breath are by no means so great as those produced in the tidal air, since the latter forms only a small proportion of the total air in the lung alveoli. Thus, at the end of a normal expiration, the alveoli still contain 2500 c.c. of gases. In inspiration 360 c c. atmospheric air are taken into this space and mixed with the 2500 c.c. already there. The ' ventilation coefficient ' in quiet breathing is therefore only one-seventh, and the change in the oxygen and carbon dioxide content of the alveolar air produced by this access of 360 c.c., will amount to less than one-half per cent. This is illustrated by the following figures from Haldane and Priestley,* giving the alveolar content in carbon dioxide at the end of inspiration and at the end of expiration respectively.

ALVEOLAR CO_2 PER CENT.

Individual	Alveolar CO_2 at end of inspiration. (Mean of twelve observations) per cent.	Alveolar CO_2 at end of expiration per cent	Mean
J. S. H.	5·54	5·70	5 62
J. G. P.	6·17	6·39	6·28

* HALDANE and PRIESTLEY. *J. Physiol.*, 1904, **32**, 222.

We can thus speak of an average composition of alveolar air, which, in spite of the constant ventilation, differs from the external air in containing an excess of carbon dioxide and a deficit of oxygen.

THE BLOOD GASES

It was shown by Magnus, in 1837,* that the blood passing to the lungs contained more carbon dioxide and less oxygen than that passing away from the lungs. This proved that combustion does not occur in the lungs, as Lavoisier had suggested,† but that the blood acts simply as a carrier of the oxygen from the lungs to the tissues, and of the carbon dioxide from the tissues to the lungs. We thus learnt to distinguish between external and internal respiratory processes. If any further proof is required that it is the tissues themselves which utilise oxygen and produce carbon dioxide, we may refer to the fact that any surviving tissue, quite free from blood, can be shown to absorb oxygen and give out carbon dioxide, as may readily be demonstrated by the use of the microrespirometer (p. 146). A consideration of the process of external respiration necessitates an investigation of the manner in which gases are held by the blood and of the factors which are responsible for the transfer of oxygen and carbon dioxide from alveolar air to blood, and from blood to alveolar air.

If blood be exposed to a vacuum, preferably at body temperature, it froths up, and the whole of its contained gases are given off.

In this manner, about 60—70 c.c. of mixed gases may be obtained from 100 c.c. of blood; it consists of oxygen, carbon dioxide and nitrogen (with argon in insignificant quantities). The nitrogen forms only about 1 volume per cent. and is present in the same proportion in both arterial and venous blood. The amounts of oxygen and carbon dioxide in these two kinds of blood differ, however, within wide limits. The following Table ‡ represents the average composition of the gases obtained from arterial and venous bloods in man:

From 100 vols.	May be obtained Of oxygen	Of carbon dioxide	Of nitrogen
Of arterial blood	19·0 ± 0·49 vols.	49·9 ± 0·51 vols.	0·9 vols.
Of venous blood	10·6 ± 0·36 ,,	57·8 ± 0·35 ,,	,, ,,

Measured at 760 mm. and 0° C.

Simple evacuation, though a standard method, is very laborious, and demands relatively large amounts of blood.

The modern methods, based on the expulsion from the blood of its combined oxygen by the use of ferricyanide (p. 530) and of its combined CO_2 by addition of an acid, enable accurate determinations to be made on 0·5 to 2·0 c.c of blood, and so can easily be used on human blood drawn by puncture of an artery or vein, as well as on the blood of animals. One valuable apparatus is that of Van Slyke (p. 516) and another is the Haldane apparatus (Fig. 478).

In connection with our present purpose we need to know, not only the *oxygen content* of samples of blood collected under specified conditions, but often also the *oxygen capacity* of the same blood, *i.e.* the amount of oxygen it will contain when fully saturated. This, of course, depends upon the hæmoglobin content of the particular sample of blood. Oxygen capacity is determined as described on p. 515. If we know the oxygen content

* MAGNUS. *Ann. d. Physik.* 1837, **40**, 583; 1845, **66**, 177.
† SEGUIN and LAVOISIER. *Mém. Acad. Sciences*, 1790, 77.
‡ After data from LOONEY and JELLINEK. *Am. J. Physiol*, 1937, **118**, 225.

and capacity we can then calculate the *percentage oxygen saturation* of the particular sample, which $= \frac{\text{content}}{\text{capacity}} \times 100$, *e.g.* if a sample has an oxygen capacity of 20 volumes per cent. and an actual content of 10 volumes per cent., then it is only 50 per cent. saturated, *i.e.* it has a *deficit* of 10 volumes per cent. so that in order to saturate that sample a further 10 c.c. of oxygen per 100 c.c. would be taken up.

It is easy, on any given sample of blood, to determine at one time the oxygen content, percentage saturation and capacity, by the use of Haldane's apparatus. For this

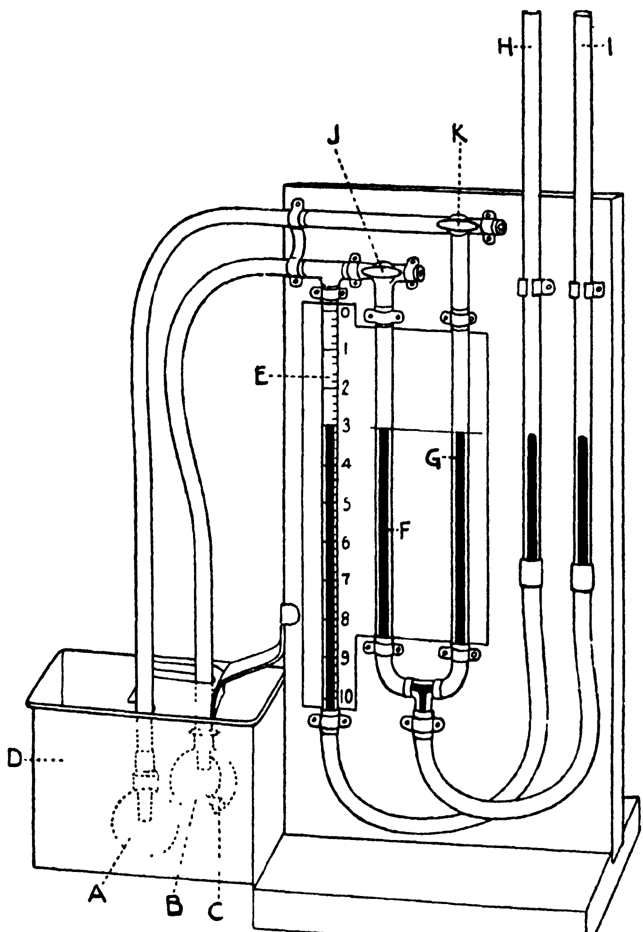

FIG. 478. Haldane's Blood-gas Apparatus.

purpose, 2 c.c. of very dilute ammonia solution are placed in flask B and a few milligrams of saponin added; then 2 c.c. of blood are run beneath the surface of the alkali, and the flask corked and immersed in the water bath D until its temperature is constant. The flask A contains 4 c.c. of water, and is similarly corked, and the T-taps J and K left open to the air. The levelling tube I is adjusted to bring the water in F and G to the marks, and H to bring that in E to a suitable level, and then taps J and K are turned so that the T-bores lie thus: ⊣. The burette E is now read, and the flask B agitated for a few minutes so that the layer of blood is mixed with the alkali, laked, and fully saturated with oxygen. After again equalising temperatures, the levels in F and G are adjusted by the levelling tubes H and I. The blood has now absorbed the amount of oxygen necessary to saturate it; this volume ('the deficit') is read off on the burette E and

corrected for temperature and pressure; call it D c.c. Flask B is now opened, ferricyanide added to the tube C, the flask closed, and the oxygen capacity of the now fully saturated blood determined by adding the ferricyanide. Call it S c.c. After making certain minor corrections * we can then calculate the original oxygen content C:

$$C = S - D$$

and the percentage saturation, P

$$P = \frac{S - D}{S} \times 100$$

For instance, suppose that the oxygen capacity was 17·4 c.c. per 100 c.c. of blood, and the deficit was 0·92 c.c. per 100 c.c. blood, then the blood as taken contained 17·4 − ·92 = 16·48 c.c. oxygen per 100 c.c., while the percentage saturation would be $\frac{16{\cdot}48}{17{\cdot}40} \times 100 = 94{\cdot}7$.

LAWS OF SOLUBILITY OF GASES IN LIQUIDS. The laws which govern the amounts of gases held by the blood are investigated by finding the amount of gas which the blood will take up when exposed to different pressures of the gas. If a gas is in simple solution, the amount of it dissolved varies directly with its partial pressure. When only one gas is present, the whole pressure is due to that one gas. Thus, if water dissolves a certain quantity of a gas at a given temperature and pressure, it will take up twice as much if the pressure of the gas be doubled. The absorption coefficient of a liquid for a gas at a given temperature is expressed by the number of cubic centimetres of gas, measured at normal temperature and pressure (0° and 760 mm. Hg), which will be taken up at the particular temperature by 1 c.c. of the liquid when the gas is at a pressure of 760 mm. Hg. The absorption coefficient diminishes with rise of temperature. The following Table represents the absorption coefficients for some important gases in water at various temperatures between 0° and 40° C.:

Temperature	Oxygen	Carbon dioxide	Carbon monoxide	Nitrogen †
0	0·0489	1·713	0·0354	0·0239
10	0·0380	1·194	0·0282	0·0196
20	0·0310	0·878	0·0232	0·0164
30	0 0262	0·665	0·0200	0·0138
40	0·0231	0·530	0·0178	0·0118

From this Table we see that 100 c.c. of water at 0° C. will absorb 4·89 c.c. oxygen at one atmosphere pressure. If the pressure be altered, the amount of oxygen dissolved will vary proportionately, *e.g.* if the pressure is reduced to one half an atmosphere, the volume of gas absorbed, when measured at N.T.P., will be 2·45 volumes. If, therefore, we plot out the absorption of the gas on a graph, of which the ordinates represent the amount of gas dissolved, and the abscissæ the different pressures of the gas, we shall get a straight line. The amount absorbed depends on the partial pressure of the particular gas considered, and is not altered by the presence of other gases at the same time. The normal pressure of the whole atmosphere is 760 mm. Since the atmosphere consists roughly of four parts of nitrogen with one part of oxygen, the atmospheric pressure is due, as to one-fifth to the oxygen, and as to four-fifths to the nitrogen. If we shake up water at 0° C. with the atmospheric air at the ordinary pressure, 100 c.c. of water

* HALDANE. "Respiration," p 410. (Yale Press, 1922.)

† Atmospheric "nitrogen" contains 1 185 per cent. argon, and this mixture is slightly less soluble than pure nitrogen, *e.g.*, 0 0134 at 30° C

will absorb 4·89 c.c. × $\frac{1}{5}$ of oxygen, and of nitrogen 2·39 c.c. × $\frac{4}{5}$. We may therefore extend our statement as to the solubility of gases in liquids, and say that *the amount of gas dissolved in a liquid is proportional to the partial pressure of the gas.*

When water is saturated with a gas at a given pressure, a state of equilibrium exists between the gas dissolved and the gas in contact with the fluid. In this equilibrium, the number of molecules of the gas entering the fluid in unit time is equal to the number of molecules of the gas leaving the fluid. If we remove the liquid after saturation, say, at one atmosphere, to a vessel where it is in contact with the same gas at a pressure of half an atmosphere, the liquid will give off gas until the amount left in solution is diminished to one-half. The gas dissolved in a liquid thus has a pressure or *tension* tending to make it escape from the liquid. The only way in which we can measure this tension is by finding what pressure of that gas is in equilibrium with the liquid. Thus, if we take some water containing carbon dioxide in solution, divide it into two parts, and shake up one part with a gaseous mixture containing 4 per cent. of carbon dioxide and the other part with a mixture containing 5 per cent. of carbon dioxide, and find that the solution loses gas to the former and takes up carbon dioxide from the latter, we may conclude that the tension of carbon dioxide in the original fluid was something between 4 and 5 per cent. of an atmosphere. It is by similar means that the tensions of gases in the blood are measured, the instruments for this purpose receiving the name of *aerotonometers.*

The solvent power of water for gases is diminished if the water contains solid substances in solution. Blood plasma, or blood corpuscles, will therefore have a smaller solvent power for gases than has pure water. Bohr showed that the depression of solubility caused by the presence of proteins or salts in solution is similar for all gases. The absorption coefficient of blood plasma for gases is reduced to 97·5 per cent. of that for pure water, and of blood to 92 per cent., that of the blood corpuscles being as low as 81 per cent. owing to their smaller content of free water. We may thus reckon the absorption coefficient (for physical solution) of blood plasma, blood and blood corpuscles for oxygen, nitrogen and carbon dioxide.

	Oxygen		Nitrogen		Carbon dioxide	
	15°	38°	15°	38°	15°	38°
Blood plasma .	0·033	0·023	0 017	0 012	0·994	0·541*
Blood . .	0 031	0·022	0 016	0·011	0·937	0·511
Blood corpuscles .	0·025	0·019	0 014	0·010	0·825	0·450

THE CARRIAGE OF OXYGEN BY THE BLOOD

The oxygen dissociation curve. From the above Table we see that 100 volumes of blood at 38° C. might contain 2·2 c.c. of oxygen in solution, if the blood had been exposed to oxygen at a pressure of one atmosphere. The blood in the lungs is, however, exposed to air which contains only about one-sixth of its volume of oxygen, so that the total amount of oxygen present in solution in arterial blood cannot be more than one-sixth of 2·2, *i.e.* about 0·36 c.c. per cent. But blood saturated with oxygen by shaking with air contains nearly twenty volumes per cent. of oxygen, so that most of the

* Van Slyke and his collaborators have estimated the solubility co-efficient of CO_2 in blood plasma at 38° C. as 0·510 instead of 0 541 as given by Bohr.

oxygen cannot be in solution, but must be in some form of combination with some of the constituents of the blood. Of this oxygen, practically the whole is contained in the red blood corpuscles, in combination with hæmoglobin, the plasma containing no more than can be accounted for by simple solution.

If oxygenated blood, or a solution of oxyhæmoglobin, be subjected to gradually diminishing oxygen pressure at the temperature of the body, *e.g.* by placing it in a partial vacuum, or by replacing the gas in contact with it by nitrogen, very little oxygen is given off until the partial pressure of the oxygen is diminished to about 30 mm. Hg (Fig. 479). At this point a rapid evolution of oxygen begins, and continues at falling pressure until, at 0 mm. pressure, all the oxyhæmoglobin is converted into hæmoglobin. The same observation may be made in the reverse direction. If a solution of reduced hæmoglobin be exposed to gradually increasing pressures of oxygen, it will be found that the greatest absorption takes place between 0 and 30 mm. Hg. After this point, the oxygen is more slowly absorbed up to the point of complete saturation.

Since there is no direct proportion between the partial pressure of the oxygen and the amount of it absorbed, it is evident that the oxygen combines reversibly with hæmoglobin to form an unstable compound, and that this is not a mere question of solution. This is further proved by the fact that we can displace the oxygen (O_2) from the oxyhæmoglobin by equal volumes of CO or NO.

The relation between the partial pressure of oxygen and the proportion of oxyhæmoglobin present (or 'percentage saturation') when equilibrium is reached under varying conditions, can be investigated in the following way (Barcroft):

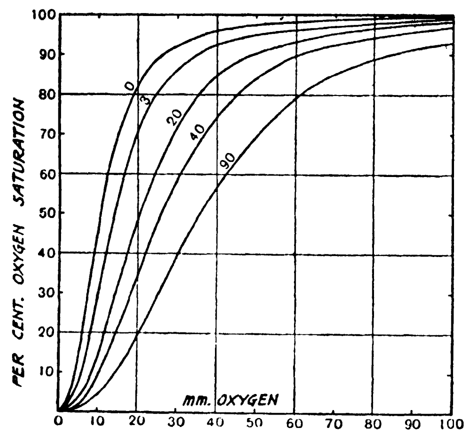

FIG. 479. Oxygen Dissociation Curves of Human Blood, exposed to pressure of 0, 3, 20, 40 and 90 mm. of CO_2. (BARCROFT.)

A cylindrical glass vessel of about 300 c.c. capacity is filled with a gaseous mixture of known composition, containing oxygen. Into it are introduced 2 or 3 c.c. of blood or of hæmoglobin solution. It is then stoppered and immersed in a horizontal position in a water bath at a constant temperature, and slowly revolved. In this way the blood is continually spread in a thin layer over the sides of the vessel. At the end of a quarter to half an hour it will have attained equilibrium with the gaseous mixture, and a sample may be drawn off for determination of its percentage oxygen saturation. A sample of the gas is also analysed to find the partial pressure of its components. The results, plotted as in Fig. 479, where abscissæ are pressures of oxygen in mm. Hg, and ordinates are percentage saturations, form what is called the '*dissociation curve*' of oxyhæmoglobin. It tells us what percentage of the hæmoglobin is saturated with oxygen at each partial pressure of that gas.

The dissociation curve approximately fits the equation (Hill's equation *): —

$$\frac{y}{100} = \frac{Kx^{2\cdot5}}{1 + Kx^{2\cdot5}},$$

* A. V. HILL. *J. Physiol.*, 1910, **40**, Proc. iv.

where x = oxygen pressure, y = per cent. saturation of the Hb, and K the dissociation constant. Hence if one point is actually determined, the rest of the curve can be approximately calculated. The theoretical significance of the curve is doubtful.* The fact that the curve is not a rectangular hyperbola, though it is somewhat similar, is important. The S-shaped part which it shows at the lower oxygen pressures means that with falling oxygen pressures the hæmoglobin gives up its oxygen more easily than the rectangular hyperbola would require.

Barcroft showed that the dissociation curve of oxyhæmoglobin is largely altered by slight variations in the fluid in which the hæmoglobin is dissolved. The most important of these conditions are (1) the saline content of the fluid, (2) the reaction of the fluid.

The differences between the dissociation curves of blood and of oxyhæmoglobin solution were shown by Barcroft and Camis to be largely dependent on the saline content of the solution.

More important is the effect of change of hydrogen-ion concentration, or so-called 'Bohr effect.' In Fig. 479 is represented the influence of varying tensions of carbon dioxide on the dissociation curve. It will be seen that the more acid the blood, the less firmly is its oxygen held. In other words, carbon dioxide, or other acid, tends to expel oxygen from the blood. The normal tension of CO_2 in arterial blood is about 40 mm. Hg. At 150 mm. oxygen pressure the blood is practically saturated with oxygen, whatever (within physiological limits) the pressure of the carbon dioxide ; but at lower pressures of oxygen, the pressure of carbon dioxide makes a considerable difference to the oxygen saturation. Thus, at an oxygen pressure of 20 mm. Hg the amount of oxyhæmoglobin formed is 70 per cent. when the carbon dioxide pressure is 3 mm., whereas at a pressure of carbon dioxide of 40 mm. the degree of saturation of the hæmoglobin is only 33 per cent. In consequence of this fact, in the tissues where the carbon dioxide tension is high, the oxyhæmoglobin will be dissociated with greater ease, so that oxygen will readily be set free where it is most wanted.

Another important factor which affects the oxyhæmoglobin dissociation curve is, of course, the temperature at which equilibrium is effected. Rise of temperature, like rise of H-ion concentration, flattens out the curve, *i.e.* rise of temperature facilitates the removal of oxygen from oxyhæmoglobin.†

Uptake of Oxygen in the Lungs. We are now in a position to understand how the oxygen is taken up by the blood, as it circulates round the pulmonary alveoli. Arterial blood, such as that which fills the pulmonary veins and the systemic arteries, is very nearly (viz. about 96 per cent.) saturated with oxygen (*e.g.* it contains about 19 volumes out of a possible 20 volumes per cent.). Venous blood, as passed to the lung, is about 66 per cent. saturated (*i.e.* it contains about 13 volumes of oxygen per cent.). The oxygen tensions are about 100 mm. for arterial blood, and 40 mm. for venous blood, *i.e.* at a tension of 100 mm. the blood is nearly saturated. The pressure of oxygen in the alveoli is normally definitely above this. If we take the oxygen pressure in the alveolar air at $\frac{1}{7}$ of an atmosphere, it will still be something over 100 mm. Hence the venous blood brought to the alveoli by the pulmonary artery will, on coming into intimate contact with alveolar air, take up oxygen from it to saturation, or to a point not far removed from it.

Passage of Oxygen to Tissues. The blood, thus laden with oxygen, is sent from the left side of the heart to all parts of the body. It must be remembered that, neither in the lungs nor in the tissues, does the hæmoglobin come in actual contact with the source of the oxygen, or with the cells which

* McLean. *Physiol. Rev*, 1938, **18**, 495.
† Barcroft and King. *J. Physiol.*, 1909, **39**, 374.

it is to supply. In both cases, the interchange is effected through the intermediation of the plasma and, in the tissues, of the tissue fluid as well. Since the tissue elements are constantly using up oxygen, they absorb any oxygen that is present in the surrounding fluid. There is, in consequence, a descending slope of oxygen tensions from red blood corpuscle through plasma, vessel wall, tissue fluid and tissue element and (assuming that all the media are permeable to oxygen) the oxygen is bound to follow this gradient. The cell draws from the tissue fluid, and the tissue fluid from the plasma, so that the oxygen tension in the plasma sinks. The immediate result is an evolution of oxygen from the oxyhæmoglobin, and the oxygen set free is taken up by the plasma, to be in turn passed on to the tissue fluid and cells.

The rate of passage of oxygen out of the capillaries into the tissue cells must thus be proportional to the difference of oxygen tension between the capillaries and the cells. If Pb is the oxygen pressure in the capillary blood and Pt that in the tissues, the rate of flow of oxygen from capillaries to tissues must be proportional to Pb — Pt. If the blood is to lose oxygen during the whole of this passage through the tissues, we may take for Pb the tension of the oxygen in the venous blood as it leaves the tissues. This is, say, 30 mm. Hg. Pt, the tension of oxygen in the tissues, may be determined in various ways,* but is usually considerably lower than Pb.

Krogh † determined the rate of diffusion of oxygen through different thicknesses of various tissues (connective tissue, muscle, etc.) under varying pressures. Then, partly on the living muscle, partly on injected specimens, he estimated the relation of the number and size of the capillaries to the intercapillary muscular tissues. He found that extremely small pressure differences were necessary to supply the muscle fibres with oxygen. Some of his results on the guinea-pig are shown in the accompanying Table.

Guinea-pig muscle	c c O_2 per minute per 100 c c tissue	Capillaries per mm²	Pb — Pt mm Hg.	Total surface capillary in 1 c c. muscle	Capacity of capillaries in 100 c c. muscle
Rest	0·5	31	45	3 cm.²	0·02 c.c.
	0 5	95	12	8 ,,	0 06 ,,
	0·5	270	3	32 ,,	0·3 ,,
Massage	0 5	1400	0 04	200 ,,	2·8 ,,
Work	5	2500	1·4	390 ,,	5·5 ,,
Maximum circulation	10	3000	1·2	750 ,,	15 ,,

The first thing that strikes us in this Table is the enormous difference between the capillary circulation of resting and that of active muscle. In the resting muscle the majority of the capillaries are empty and collapsed, so that large areas of muscle intervene between the few capillaries in which the circulation of blood is proceeding. Under these conditions, the pressure difference necessary to supply the total oxygen consumed by the muscle, *e.g.* 45 mm. Hg, may fall below the venous oxygen tension, so that in parts of the muscle the oxygen tension may be zero. After massage, a number of capillaries open; the number is still further increased by work, so that there may be a hundredfold increase in the number of capillaries in every square millimetre of a cross-section of the muscle. Under these conditions, the passage of oxygen from the capillaries is so facilitated that the oxygen

* VERZÁR. *J. Physiol.*, 1912, **45**, 39

† KROGH. "The Anatomy and Physiology of the Capillaries." (Yale Press, 1929.)

pressure in the muscle tissues becomes practically equal to that of the blood. It would appear that, so far as the supply of oxygen to the muscle is concerned, the increase in the capillary area during muscular exercise is far ahead of the actual needs of the muscle.

Under normal circumstances the blood never stays in the capillaries long enough to lose the whole of its oxygen. The fraction of the arterial oxygen abstracted, which is called the *coefficient of oxygen utilisation*, varies from tissue to tissue, and from time to time. In states of activity, the venous blood may be more than usually deoxygenated, but generally not more than 0·4 of the arterial oxygen is utilised. If, however, the further supply of oxygen

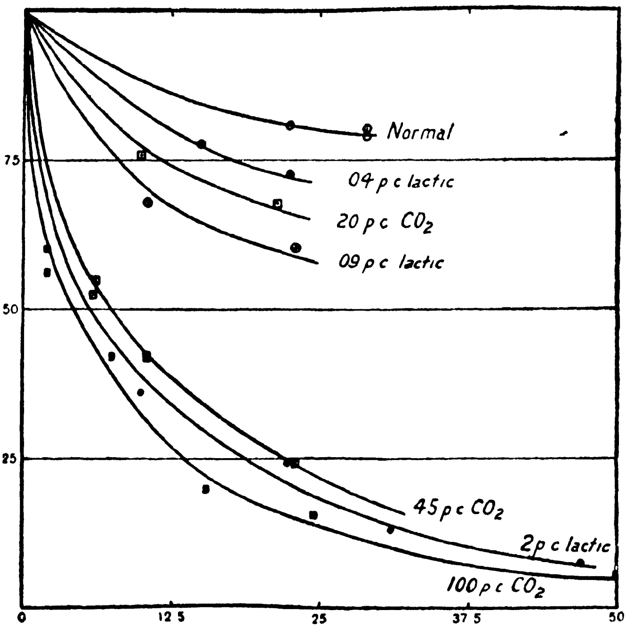

FIG. 480. Curves showing the Rate at which Arterial Blood is Reduced on Bubbling through it a Gas free from Oxygen, and the effect on the Rate of the Presence of CO_2 and of Lactic Acid.

Ordinates = percentage saturation of oxyhæmoglobin. Abscissæ = time in minutes. (MATHISON. *J. Physiol.*, 1911, **43**, 347.)

to the blood be prevented, as in asphyxia, the last traces of oxygen may disappear from the blood.

As a result of the oxidative changes in the tissues, carbon dioxide is produced, and the tension of this gas in the tissues therefore rises.

As Barcroft has pointed out,* in cold-blooded animals the hæmoglobin is different in its properties from that of warm-blooded animals, being less affected by temperature changes. In them, too, the dissociation of oxyhæmoglobin with the setting free of oxygen must be largely conditioned by the rise of carbon dioxide tension in the tissues, since at the normal temperature of these animals the evolution of oxygen from hæmoglobin would be slow.

The alteration in reaction of the blood, caused by a rise in CO_2 tension or by the presence of small amounts of lactic acid, markedly quickens the rate at which oxyhæmoglobin gives up its oxygen, as is shown in Fig. 480.

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* BARCROFT. *Physiol Rev.*, 1925, **5**, 596.

THE CARRIAGE OF CARBON DIOXIDE BY THE BLOOD

Carbon dioxide is soluble in water, and therefore in blood, which at 38° C., when exposed to a pressure of 40 mm. CO_2, *i.e.* about that of arterial blood, would hold, in a state of *physical* solution, about 2·7 volumes of CO_2 per 100 c.c. of blood. But blood, as drawn from the body, may contain as much as 60 volumes per cent. of carbon dioxide, held in such a state as to be removable in a vacuum, and we have now to inquire why it is that blood is capable of holding such large amounts of carbon dioxide. A further fact is that this carbon dioxide can also be expelled from it by the addition of strong acids. This suggests at once that the carbon dioxide is held in the blood as a bicarbonate or carbonate, from which union it is expelled by acid, or when the blood is exposed to a vacuum. It should further be noted that, after all possible carbon dioxide has been drawn from blood by exposure to a vacuum, no more can be driven off on addition of a strong acid. One method of expulsion is as good as the other, so far as the total yield of CO_2 from whole blood is concerned.

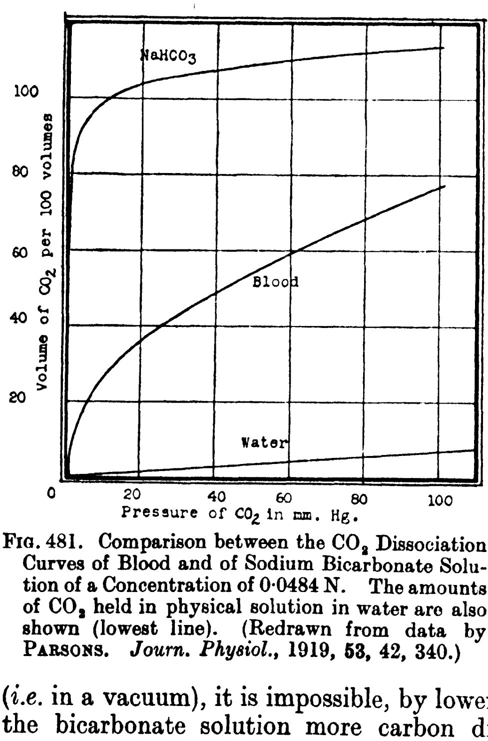

Fig. 481. Comparison between the CO_2 Dissociation Curves of Blood and of Sodium Bicarbonate Solution of a Concentration of 0·0484 N. The amounts of CO_2 held in physical solution in water are also shown (lowest line). (Redrawn from data by Parsons. *Journ. Physiol.*, 1919, **53**, 42, 340.)

(1) **Carriage as Bicarbonate.** Valuable information has been obtained by the study of the CO_2 dissociation curves of blood, and of somewhat similar chemical systems. These are made by saturating samples of the fluid with a series of gas mixtures of varying CO_2 pressure, and then determining the volume of CO_2 which can be expelled from the fluid by addition of a strong acid. If we compare the CO_2 dissociation curve of blood with that of a solution of sodium bicarbonate containing the same amount of available base (0·0484 N.), it is evident, as is shown in Fig. 481, that there is a very great difference between them. For, whereas all the carbon dioxide is removed from the blood when the pressure of CO_2 is reduced to zero (*i.e.* in a vacuum), it is impossible, by lowering the pressure, to remove from the bicarbonate solution more carbon dioxide than corresponds to its conversion to carbonate, according to the equation—

$$2NaHCO_3 = Na_2CO_3 + CO_2 + H_2O;$$

i.e. from an aqueous bicarbonate solution only half as much carbon dioxide can be expelled by a vacuum as by addition of a strong acid :—

$$2NaHCO_3 + 2HCl = 2NaCl + 2CO_2 + 2H_2O.$$

The carbon dioxide in blood is chiefly bound in the plasma in the form of sodium bicarbonate. This is shown by the fact that the amount of carbon dioxide which can be expelled from the plasma by addition of a strong acid

agrees with the amount of base found by titration, and further, all this bicarbonate can be removed as such by dialysis. If plasma is saturated with carbon dioxide at atmospheric pressure, practically all the available base of the plasma will be present in the form of sodium bicarbonate. The amount of bicarbonate present in the plasma under any circumstances can readily be determined, and is called the "*alkali reserve.*" *

If we examine the dissociation curve of blood plasma, we find that separated plasma behaves less like blood than like an aqueous bicarbonate solution (Fig. 482). This is confirmed by the well-known fact that, although all the CO_2 can be removed from blood by a vacuum, this cannot be done with blood plasma; after plasma has been thoroughly pumped out *in vacuo*, addition of acid can still set free carbon dioxide from it. It is clear then, that if the carbon dioxide *is* fixed in the form of bicarbonates in the blood, there

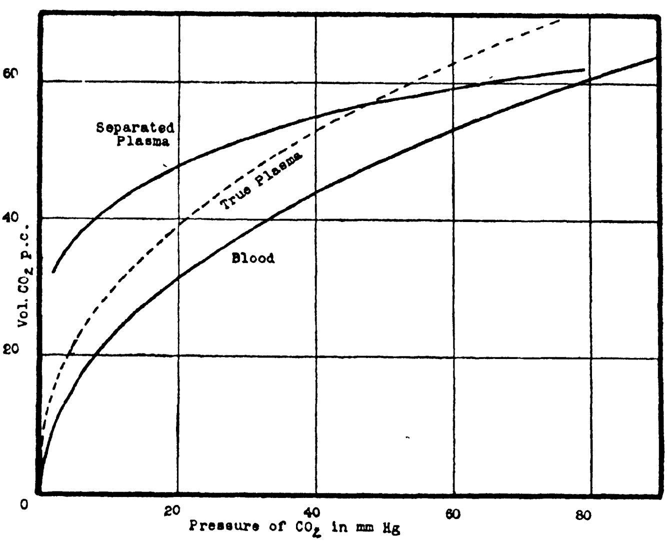

FIG. 482. Comparison between the CO_2 Dissociation Curves of Blood, and of Separated Plasma and true Plasma from the same Blood. (Redrawn from data by JOFFE and POULTON. *J. Physiol.*, 1920, **54**, 129.)

must also be in the blood some substance which, while not interfering with the taking up of carbon dioxide by the base when the blood is exposed to increasing pressures of carbon dioxide, will yet act as an acid does, and drive out carbon dioxide, ultimately to completion, as the partial pressure of CO_2 falls. *This substance must be contained in the corpuscles.* It is the corpuscles which *in vacuo* act like an acid, expelling carbon dioxide. This can be proved by the simple experiment of mixing thoroughly well-evacuated plasma with corpuscles, when CO_2 is at once evolved on further evacuation.

It is also shown by the comparison of true plasma with separated plasma. The name '*true plasma*' is given to plasma drawn direct from the blood, without any change in its gas content. Specimens of true plasma are obtained by centrifuging the blood under a layer of liquid paraffin, to hinder exchange of gases at the surface, and then pipetting off the plasma layer. This represents the plasma which was in a state of equilibrium with the corpuscles of the blood when this was centrifuged.

* Strictly speaking, the "alkali reserve" is defined as the volume per cent. of CO_2 yielded by the plasma on treatment with acid after the plasma has been previously brought into equilibrium with a gas mixture containing CO_2 at 40 mm. pressure (alveolar air).

Now, if blood be exposed to gas mixtures containing different pressures of carbon dioxide, as in making a CO_2 dissociation curve, it is a simple matter to separate off samples of true plasma corresponding to each pressure of CO_2, to determine their bicarbonate content, and thus to obtain a curve which may be called the dissociation curve of true plasma. Such a curve represents the carbon dioxide content of plasma which at each point is in equilibrium with the corpuscles. When this is done, it at once becomes evident that the true plasma behaves in a way entirely different from the separated plasma discussed above. Fig. 482 illustrates this difference.

The true plasma shows a curve which has much the same slope as that for blood, but at a higher level; the plasma of blood, when in equilibrium with its corpuscles, has sometimes a higher percentage bicarbonate content not only than separated plasma, but even than the whole blood. This is because the carbon dioxide-fixing power of corpuscles is less than that of plasma.

The explanation of the way in which the corpuscles confer upon the plasma the power of fixing CO_2 by the formation of bicarbonate was given by experiments by Zuntz * and by Hamburger.† The latter observer showed that when carbon dioxide is added to blood the following changes occur:—

(1) The bicarbonate content of the plasma is increased.

(2) The chloride content of the plasma is diminished.

(3) The chloride content of the corpuscles is increased.

The cations (base) of the plasma and corpuscles are unchanged.

The reason why blood can carry much more carbon dioxide than water is that the H˙ ions formed in the reactions:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^{\cdot} + HCO_3'$$

are all mopped up by buffers in the blood, so that the reaction is accelerated from left to right. In consequence of this the CO_2 carrying power of the blood runs parallel to its buffering power. Some of the buffers of the blood are present in the corpuscles and some in the plasma: in the corpuscles we have hæmoglobin, which is the chief buffer of the blood, while in plasma we have small amounts of phosphate, and the plasma proteins.

Since the red cells are freely permeable to CO_2, and since hæmoglobin is quantitatively the most important blood buffer, it follows that when carbon dioxide enters the blood, far more HCO_3' ions are formed in the corpuscles than in the plasma. The red cells are also easily permeable to HCO_3' ions, and so, since there is a gradient of HCO_3' from cells to plasma, this ion will tend to migrate out again into the plasma. But the envelope of the red cell is not permeable to cations, and so the HCO_3' ions cannot be accompanied out by the positively charged ions, such as $K^{\cdot}$. Hence, owing to the tendency of the HCO_3' to migrate, the cell becomes positively charged, and this, so to speak, sucks into it negatively charged ions, such as Cl', which are abundant in plasma. This offers an explanation of the so-called Hamburger interchange, viz., that as CO_2 is added to blood the plasma bicarbonate increases and the plasma chloride diminishes. The advantage of this is that it enables the excellent buffering power of the corpuscles to be shared out with the poor buffering power of the plasma.

THE EFFECT OF OXYGENATION ON CARBON DIOXIDE CARRIAGE. It was found by Christiansen, Douglas and Haldane ‡ that reduced blood has a greater carbon dioxide capacity than oxygenated blood (Fig. 483).

In other words, oxygen tends to displace carbon dioxide from the blood, just as carbon dioxide is known to displace oxygen. They explained this

* ZUNTZ. *Hermann's Hdbk. d. Physiol.*, **4** (ii.), 77 (1882).

† HAMBURGER. *Arch. f. Phys.*, 1892, 513; 1893, Suppl. 153; *Bioch. Ztschr.*, 1918, **86** 309.

‡ CHRISTIANSEN, DOUGLAS and HALDANE. *J. Physiol.*, 1914, **48**, 244.

phenomenon on the hypothesis that reduced hæmoglobin is a weaker acid than oxyhæmoglobin, *i.e.* its salts mop up $H^{\cdot}$ ions more easily.

The importance of this observation lies in the fact that during its passage along the systemic capillaries, not only is carbon dioxide added to the blood, but also oxygen is withdrawn from it, and so the buffering power of the blood is raised, thus enabling more CO_2 to be taken up and *vice versâ* in passing the lungs.

It is of interest in this connection to attempt to form an opinion of the carbon dioxide absorption curve of blood under the conditions which prevail in the body. Such a curve (for an R.Q. of about 0·8) is shown in Fig. 483, in which portions of the carbon dioxide curves for fully reduced and fully oxidised blood are shown on a large scale. The curve AVB represents the

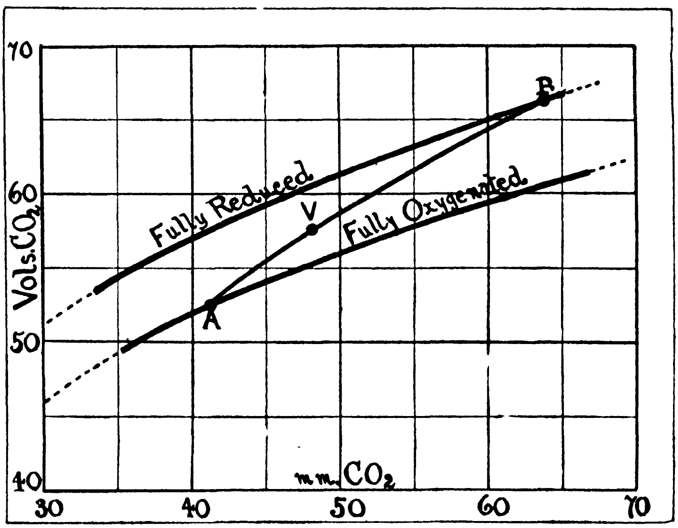

FIG. 483. CO_2 Dissociation Curves of Fully Reduced and Fully Oxygenated Human Blood (only part of the curve is shown). A V B is the physiological curve, along which the CO_2 dissociation operates in the body. (Redrawn from CHRISTIANSEN, DOUGLAS and HALDANE.)

physiological carbon dioxide absorption or dissociation curve. At the point A, which may be called the '*arterial point,*' since it represents the condition in arterial blood, the fully oxygenated blood at 14 mm. CO_2 contains 52·5 volumes CO_2 per cent. The point B represents the condition if the blood were fully reduced ; it would then contain, at the pressure of CO_2 of 64 mm., not 61 per cent. of CO_2 (as would be the case had there been no loss of oxygen), but 66·5 per cent. Usually the blood does not nearly reach the fully reduced state in passing through the capillaries, but would in most cases stop at a point about in the position indicated by V in the figure. This corresponds to a gain of 5 volumes of CO_2, and therefore a loss (since the R.Q. is assumed to be 0·8) of about 6·3 c.c. of oxygen. The point V is thus a typical ' venous point.' It will be seen that in this instance at least half the increase in CO_2 content of the blood is due to the simultaneous reduction of oxygen content.

CARBONIC ANHYDRASE. All the above facts have been found out by experiments with saturators in which blood is brought into final equilibrium with various gas mixtures—which may take from 15 to 30 minutes. The important question is, however, whether such equilibrium could occur in

the short time of one or two seconds during which the blood is passing along the capillaries of lung or tissue. Most of the separate reactions are ionic ones, and therefore extremely rapid, but the reaction of formation of carbonic acid :

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

is easily shown to be quite slow, and if uncatalysed would, in fact, limit the whole chain of reactions to about one hundredth of the speed of those that do occur in the blood. The catalyst concerned in speeding up this reaction is an enzyme to which Roughton,* its discoverer, gave the name of *carbonic anhydrase.* Carbonic anhydrase is a protein substance which contains zinc.

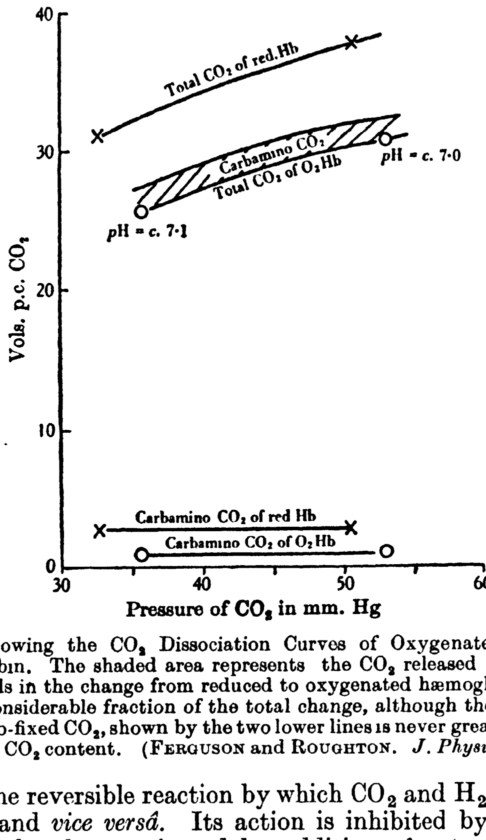

FIG. 484. Showing the CO_2 Dissociation Curves of Oxygenated and Reduced Hæmoglobin. The shaded area represents the CO_2 released from carbamino-compounds in the change from reduced to oxygenated hæmoglobin, and is seen to be a considerable fraction of the total change, although the total *amount* of carbamino-fixed CO_2, shown by the two lower lines is never great when compared with total CO_2 content. (FERGUSON and ROUGHTON. *J. Physiol.*, 1934, **83**, 87.)

It accelerates the reversible reaction by which CO_2 and H_2O combine to form carbonic acid, and *vice versâ.* Its action is inhibited by cyanide, so that after the blood has been poisoned by addition of a trace of cyanide the reactions take place much as they do in inorganic solution.

When a bicarbonate solution is acidified, we may regard its decomposition as occurring in two stages :—

$$(1)\ NaHCO_3 + HCl \rightleftharpoons H_2CO_3 + NaCl.$$
$$(2)\ H_2CO_3 \rightleftharpoons CO_2 + H_2O.$$

Acceleration of reaction (2) must therefore accelerate the rate of evolution, or uptake, of CO_2. In presence of this enzyme, which can be precipitated by an alcohol-ether mixture, the rate of evolution of CO_2 from slightly acidified

* BRINKMAN, MARGARIA, MELDRUM and ROUGHTON, *J. Physiol.*, 1932, **75**, Proc. 3; MELDRUM and ROUGHTON, *J. Physiol.*, 1932, **75**, Proc. 15.

bicarbonate solutions is greatly accelerated, *e.g.* 1 gram of the precipitate in 200 litres of solution accelerated the evolution of CO_2 five-fold. The enzyme also accelerates the converse reaction when carbon dioxide is added to the blood. It is evident that the presence of this enzyme must considerably hasten the uptake of CO_2 in the blood in the tissues and its evolution in the lungs.

Carbonic anhydrase in blood is present only in the red corpuscles. All tissues contain traces of it but the only other tissues which contain an appreciable amount of it are the pancreas and the stomach, in which it probably has a function connected with the secretion of bicarbonate and of HCl.

(2) **Carriage of Carbon Dioxide by Hæmoglobin.** It has often been suspected that hæmoglobin may be capable of combining with CO_2, the suggestion being that the hæmatin part of the molecule combined with oxygen

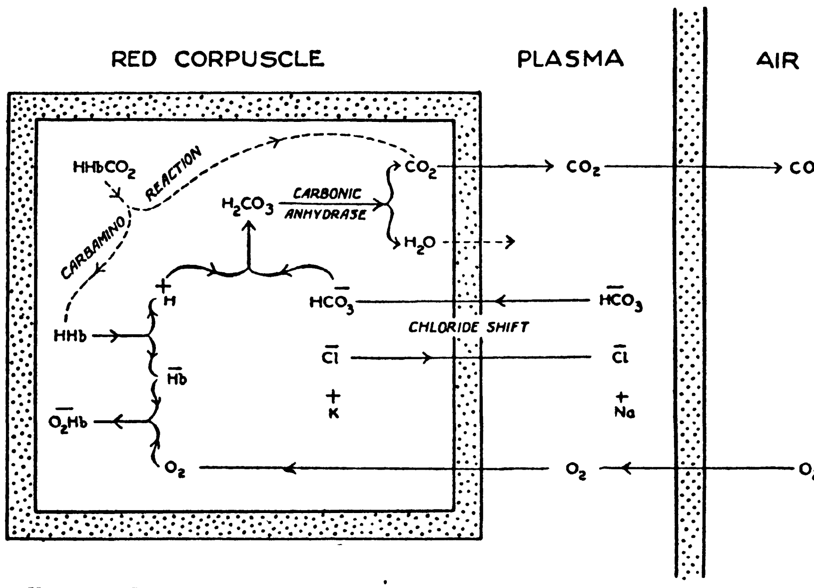

FIG. 485. Diagram of Interchanges in Corpuscle and Plasma consequent on taking up of Oxygen and expulsion of CO_2. (ROUGHTON.)

and the globin portion with CO_2. This anticipation has been confirmed by the work of Roughton and others.

When blood, poisoned with cyanide to eliminate the carbonic anhydrase, is exposed to CO_2 the gas is at first still taken up too quickly for the rate to be explicable on the ground that it was fixed in the form of bicarbonate, though the rate of uptake does ultimately slow down and accord with that supposition. This first quick absorption of carbon dioxide is due to the formation of *carbamino-compounds* by reaction with NH_2 groups in the protein molecule :—

$$CO_2 + Pr.NH_2 \rightleftharpoons Pr.NHCOOH.$$

The reaction is reversible, and is not interfered with by the presence of cyanides. The carbamino-compound, unlike bicarbonate or CO_2, is not precipitated by calcium or barium salts, and can actually be separated from blood in this way. In alkaline blood, and especially when the blood is deoxygenated, relatively more of the carbamino-compound is formed than

when the blood is less alkaline and oxygenated. Therefore, just as with the bicarbonate CO_2, oxygenation of the blood tends to expel the carbamino-bound CO_2; although the latter may only represent 5 or 10 per cent. of the total CO_2 of the blood, it is the most mobile part, and hence is responsible for a relatively large *fraction* of the difference between the CO_2 content of oxygenated and reduced blood, as Fig. 484 shows.

The various reactions in plasma and corpuscles which take place in the lungs in connection with the uptake of oxygen by, and the expulsion of CO_2 from, the blood are shown diagrammatically in Fig. 485.

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EXCHANGE OF GASES IN THE LUNGS

A fluid gives off gas to, or takes up gas from, any other medium with which it is in contact, according to the relative pressures of the gas. The question arises whether we can account by physical means for the interchange which occurs between alveolar air and blood as the latter traverses the pulmonary capillaries. In order to answer this question we must know the partial pressures or tensions of oxygen and of carbon dioxide in the alveolar air, in the venous blood coming to the lungs, and in the arterial blood leaving the lungs. If a physical explanation is to be accepted, the partial pressures and hence the flow of oxygen must be in descending order: alveolar air, arterial blood, venous blood; and those of CO_2 in the reverse order.

In the alveoli, the pressures are given by the analysis of alveolar air. The gaseous tensions in the blood, however, can only be got by finding the gaseous mixture with which the blood will be in equilibrium. If we know the amount of any gas in this mixture, we know its tension, and therefore the tension of that gas in the liquid. In all such experiments, the main difficulty is in obtaining a sufficient surface of the blood exposed to a very small volume of the gaseous mixture. A relatively small volume of gas must be used in order not to appreciably alter the blood gases. Krogh * therefore adopted the ingenious device of limiting the volume of air to a small bubble, the superficial area of which is large in proportion to its bulk. This bubble, after it has been in a stream of blood for some minutes, is transferred to a special capillary tube, in which its analysis can be carried out.

The *microtonometer* consists of the tonometer proper, and the apparatus for the micro-analysis of the gas bubble. In the latter, the measurement of the gas bubble is carried out in a capillary tube, the absorption of carbon dioxide and of oxygen being effected with potash and with pyrogallic acid. The tonometer is represented in Fig. 486. It is filled with saline solution and contains the gas bubble 2, which can be drawn up by means of the screw 4 (Fig. 486, B) into the narrow graduated tube 3 (Fig. 486, A). The blood (heparinised) from the artery or vein passes by a cannula through the tube 1,

* KROGH. *Skand. Arch. f. Physiol.*, 1908, **20**, 259.

and enters the tonometer as a fine jet. It forces its way up above the gas bubble, which is pressed a little down by the current, and kept oscillating with great rapidity.

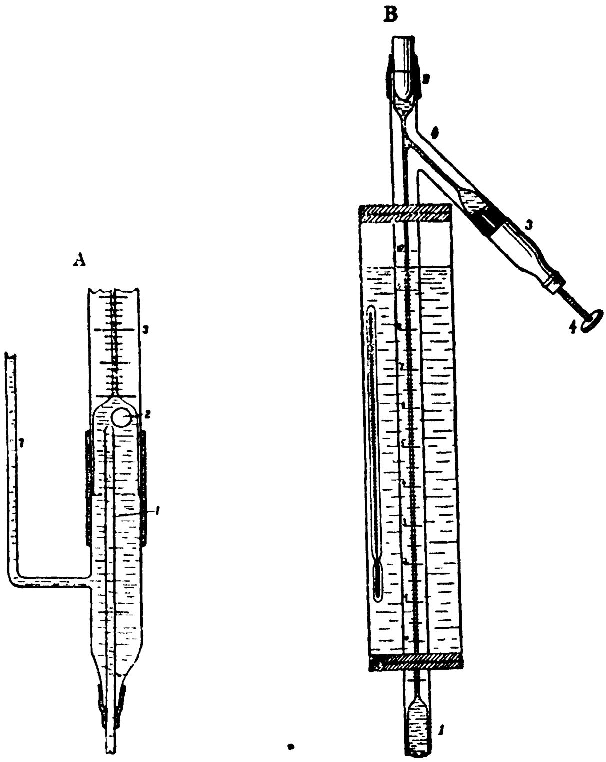

FIG 486. A. Krogh's Microtonometer. B. Upper part of Microtonometer, showing Capillary Tube into which the Bubble is returned for Measurement and Analysis.

From the tonometer, the blood flows back through the tube 7 and is reinjected into the animal if necessary. Since the total pressure of the gases in the blood is nearly always

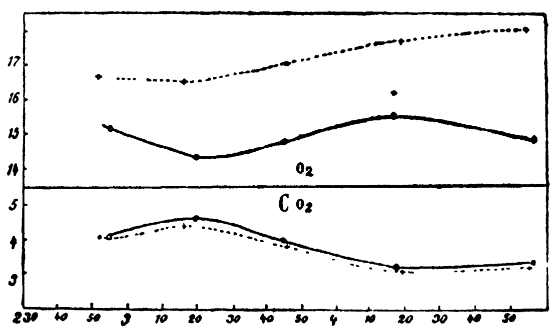

FIG. 487. Tensions, in per cent. of an atmosphere, of O_2 and CO_2 in Alveoli compared with those in Arterial Blood of Rabbit. (KROGH.)
The dotted lines represent the tensions in the alveolar air, the uninterrupted lines the tensions of the gases in the arterial blood.

ower than atmospheric pressure (see table, p. 776), it is necessary to use a device to keep the pressure in the tonometer below the arterial pressure, as otherwise the whole bubble would gradually go into solution and disappear. During the course of a tonometric experiment, the volume of the gas bubble is measured from time to

time, by drawing it up into the graduated tube, and the pressure is regulated until the volume of the bubble remains constant. After five minutes, gaseous equilibrium will have been established, and it is necessary then only to draw it up into the graduated tube and analyse it, in order to determine the tension of the gases in the blood.

In these experiments, the gaseous tensions in the alveolar air were also determined by taking samples. The results of the experiments show that the tensions of the gases in arterial blood follow closely the tensions of the corresponding gases in the alveolar air. The tension of carbon dioxide in arterial blood is either identical with, or very slightly above, the tension of the gas in the alveolar air. The oxygen tension of the blood is always lower than the alveolar oxygen tension, and the difference is generally 1 to 2—even 3 to 4—per cent. of an atmosphere. The results of a series of determinations are given in Fig. 487.

In man the relations are very similar. It has been shown by simultaneous samplings of arterial blood and alveolar air (by Haldane and Priestley's method), that samples of alveolar air collected at the end of a normal *expiration* have approximately the same carbon dioxide pressure as that of the arterial blood, both during rest * and during exercise.† At rest it is about 41 mm. Hg.

The oxygen pressures are more doubtful. If we assume that the oxygen pressure of the alveolar air at normal expiration is equal to that of the arterial blood, we obtain a figure of about 100 mm. Hg.

There is also some doubt about the exact values of the pressures of both gases in the venous blood going to the lungs. An indirect method of finding them is by employing the lung itself as an aerotonometer. By a method already described (p. 592) a suitable gas mixture is held for about fifteen seconds in the lungs, and the alveolar air has then the gas tensions of venous blood. By this means the tension of the oxygen in the venous blood was found to be about 40 mm. Hg, and that of the carbon dioxide 6 per cent. = 46 mm. Hg.

Another indirect method of finding the gas tensions in man is by reference to the oxygen or carbon dioxide dissociation curves. If we can find the oxygen and carbon dioxide contents of the bloods in question, reference to the dissociation curves will give their partial pressure. The results of such experiments as have been possible on man give support to the data obtained as above.

The partial pressures and tensions concerned in respiratory interchange are summarised in the following table :—

APPROXIMATE PARTIAL PRESSURES IN INSPIRED, EXPIRED AND ALVEOLAR AIR, AND TENSIONS IN ARTERIAL AND VENOUS BLOOD, AT BAROMETRIC PRESSURES OF 760 MM. HG.

Gas	Pressure or Tension, mm. Hg.				
	Inspired Air	Expired Air	Alveolar Air	Arterial Blood	Venous Blood (Rest)
Oxygen . . .	158·3	116	102	100	40
Carbon dioxide . .	0·3	29	40	40	46
Nitrogen . . .	596·4	568	571	570	570
Water vapour . .	5·0	47	47	47	47
Total pressure . .	760·0	760	760	757	703

* BOCK and FIELD. *J. Biol. Chem.*, 1924, **62**, 269.

† DILL, LAWRENCE, HURXTHAL and BOCK. *J. Biol. Chem.*, 1927, **74**, 313.

As the venous blood enters the lungs, there is thus a difference of oxygen pressure of about 60 mm. Hg, which will tend to cause a flow of oxygen from alveolar air to blood, and a difference of $46 - 40 = 6$ mm. Hg, tending to cause a flow of carbon dioxide from blood to alveolar air. Is this difference sufficient to account for the amounts of gases given off or taken up by the blood in its passage through the lungs? In a state of medium distension, the 3000 c.c. of air contained in the lungs has been estimated to occupy seven hundred million alveoli, each of which has a diameter of 0·2 mm., so that the total surface over which the blood is exposed to the alveolar air amounts to at least 90 square metres (about 1000 square feet). It is important to realise that the blood suddenly spreads out into a layer the thickness of which is not much more than that of one blood corpuscle, and is exposed to the air over this huge area, whence it is picked up again and collected into the pulmonary veins. So efficient a means of facilitating rapid interchange of gases between blood and air we cannot at present imitate artificially. The thickness of the tissue separating this layer of blood from the alveolar air is on the average 0·004 mm. Loewy and Zuntz calculated the rate at which oxygen would diffuse through a similar layer of tissue, and estimate that, under a constant difference of pressure of 35 mm. Hg, 6·7 c.c. of oxygen would pass in a minute through each square centimetre of the alveolar wall. Through the whole surface of the lung, this would amount to an absorption of 6100 c.c. oxygen. The oxygen actually absorbed by a man at rest amounts to about 250 c.c. per minute, so that the physical conditions allow an ample margin for increase in the consumption of oxygen; in fact, a difference of pressure of a couple of millimetres would suffice to cause a passage of the 250 c.c. per minute which is required by the resting man.* In the same way, it is easy to account for the passage of carbon dioxide in the reverse direction. This gas diffuses through a wet membrane about twenty-five times as rapidly as oxygen, so that a difference of pressure between the venous blood and the alveolar air amounting to only 0·03 mm. Hg would suffice to cause a passage outwards of the 215 c.c. of CO_2 normally expired per minute. The actual pressure difference is about 6 mm.

It is evident that the limitation to the absorption of oxygen is really given by the capacity of the hæmoglobin to combine with it. From the oxygen dissociation curve of blood we see that the amount of oxygen which can be taken up by blood in the presence of the normal tension of carbon dioxide, *i.e.* 40 mm. Hg, begins to diminish very rapidly when the pressure of the oxygen falls below 50 mm. Hg. Thus, at 40 mm. oxygen pressure, and a carbon dioxide tension of 40 mm., blood is about 75 per cent. saturated, and at 30 mm. it is only 60 per cent. saturated. Under normal circumstances, the blood leaves the lungs about 95 per cent. saturated with oxygen.† If the saturation fell to 60 per cent. we should expect to obtain evidence of failure of oxygen supply to the tissues. Probably the oxygen tension in the alveoli can sink to between 30 and 35 mm. Hg before any signs of oxygen lack make their appearance. These results were obtained by exposing a man, in a state of complete rest, to reduced pressure in an air-chamber. Under these conditions, the slightest muscular exertion would cause distress from deficient oxygen supply. The exact percentage of oxygen in the inspired air, which would give an alveolar oxygen tension of 30 to 35 mm., varies with the

* LOEWY and ZUNTZ. Die Physiol. Grundlagen der Sauerstofftherap., in Michaelis' "Die Sauerstofftherapie." Berlin, 1906.

† MEAKINS and DAVIES. *J. Path. and Bact.*, 1920, **23**, 451.

depth of respiration. Thus, with shallow respiratory movements the pressure may sink to 35 mm. Hg when the inspired air contains as much as 12 per cent. oxygen. If the movements be deeper, the oxygen content of inspired air may be reduced to 9 or 10 per cent. before respiratory distress is observed.

The view that, in the interchange of gases in the lungs, the membrane between the blood and the alveolar air plays simply a passive part is now almost universally accepted. Krogh's experiments show conclusively that the difference between the tensions in the alveoli, and in the blood, respectively, is always such as to allow of the passage by diffusion of oxygen inwards and carbon dioxide outwards from the blood. Moreover, the structure of the pulmonary epithelium lends no support to the view that it acts as a secreting membrane. In mammals the cells are of two kinds, viz. small granular nucleated cells lying in the interstices of the capillaries, and larger, extremely thin, structureless plates, *without nuclei*, covering the capillaries.

The view that the pulmonary epithelium actively secretes oxygen was held by Bohr,* and Haldane† held that, at all events under circumstances when the oxygen pressure is low in the alveoli, oxygen may be secreted, so as to attain a higher tension in the arterial blood than in the alveolar air. Thus, at high altitudes, Haldane claims that such a secretion can be demonstrated if the subject is given air containing a low percentage of carbon monoxide to breathe. Under these circumstances the blood is found to contain less CO than when shaken with the same alveolar air (containing CO) *in vitro*; *i.e.* the uptake of oxygen had been favoured, and the uptake of CO therefore impeded, in the lungs.

A reinvestigation by Barcroft,‡ of the tensions of oxygen in the blood under such conditions, and using direct methods, failed to confirm Haldane's conclusions.

ABSORPTION OF GAS FROM THE PLEURAL CAVITY. If air is introduced in sufficient amount into the pleural space to cause an artificial pneumothorax (*e.g.* in treatment of pulmonary tuberculosis) the lung on that side collapses. The air so introduced is soon altered in composition and comes into approximate equilibrium with the blood in the lung capillaries by diffusion through the visceral pleura. This result having been achieved, it might be expected that the gas would stay there indefinitely, but actually it disappears by absorption into the venous blood entering the lung capillaries. The reason for this is that, whereas the total pressure on the air in the pleural space is atmospheric, that in the venous blood (*v.* table, p. 776) is below atmospheric. Hence all the gases diffuse into the blood.

EXCHANGE OF GASES IN THE TISSUES

The considerations which determine the exchange of gases between air and blood in the lungs are also operative in determining the gaseous exchanges which take place during the circulation of the blood through the capillaries of the tissues. Blood enters the capillary network as arterial blood, and having, therefore, an oxygen pressure of the order of 100 mm. Hg and a carbon dioxide pressure of the order of 40 mm. Hg. In the tissues the oxygen pressure is lower and the CO_2 pressure higher than in the arterial blood.

The determination of the pressures of oxygen and carbon dioxide in the tissues is a matter of some difficulty. They may be approximately determined by taking the tensions of the gases in secretions, such as the urine or bile or, more generally, by injecting air beneath the skin, or into body cavities, &c., and analysing it after the lapse of a suitable interval to allow of equilibration.§ It is found to be from 45 to 55 mm. Hg for CO_2 (14 to 23 mm. for cold-blooded animals),‖ and since in venous blood it is rarely above 50 mm. Hg, there is a gradient of partial pressure of CO_2 from cells of tissues to blood,

* BOHR. *Skand. Arch. f. Physiol.*, 1890, **2**, 236.
† DOUGLAS and HALDANE. *J. Physiol.*, 1912, **44**, 305.
‡ BARCROFT *et al.* *J. Physiol.*, 1920, **53**, 450.
§ CAMPBELL. *J. Physiol.*, 1924, **59**, 1.
‖ CAMPBELL. *J. Physiol.*, 1926, **61**, 248.

viâ the tissue fluid. The gas therefore passes in that direction by simple diffusion, and the oxygen in like manner passes in the opposite direction, also by diffusion. We have already seen that the oxygen pressure may vary from one tissue to another, or in the same tissue, from time to time. It is certain, however, that the oxygen pressure in the tissues is always lower than that in the arterial blood and tends to vary inversely as the activity of the tissue. By the method mentioned above the oxygen pressure of the body cavities and of subcutaneous tissue appears to range from 20 to 45 mm. Hg. In muscle it may be lower than this, especially during activity. Under the skin, on the other hand, O_2-tension is raised during exercise, because the cutaneous vessels are dilated.

Increasing or reducing the oxygen tension in the air breathed causes a change in the same direction in both the oxygen and the CO_2-tensions in the tissues. The CO_2-pressure is increased when the oxygen-pressure is increased because CO_2 is then less readily taken up by blood and so accumulates in the tissues.*

In muscles that exhibit strong and repeated contractions there is a modified form of hæmoglobin present in the cytoplasm of the fibres. It is called *myo-hæmoglobin*, or *myoglobin*, and serves a useful purpose in facilitating the transport and storage of oxygen. It differs from blood hæmoglobin in its absorption spectrum, in having only one atom of iron per molecule, in having a relatively small affinity for CO, but especially in showing a very small Bohr effect (p. 765), and in having an oxygen dissociation curve that is a rectangular hyperbola. Consequently, it will take up oxygen much more readily at low pressures than will blood hæmoglobin, so that it will easily deprive the latter of oxygen, particularly if CO_2 is also present. It also has a higher velocity of combination with oxygen than has blood hæmoglobin, so that it can act as a quickly filled store of oxygen for the muscle fibres. It has been calculated that the myoglobin of heart muscle can hold enough oxygen for about 7 secs. of moderate activity, or about half a second of maximal activity of the heart muscle, and so should be able, with its quick rate of oxygen uptake, to keep the tissue always supplied with oxygen. It will be seen from the dissociation curves of Fig. 488 that even at the venous oxygen tension of 40 mm. the myoglobin is still 94 per cent. saturated, whereas the blood Hb is only 66 per cent. saturated. Hence the tissue could suck oxygen out even from the venous blood.

As a store of oxygen, however, the myoglobin would be of no use if it held its oxygen so firmly that the active systems of the tissue cells were unable to withdraw it. It will be seen from Fig. 488 that this is not the case, for the tissue oxidases can function at oxygen pressures of 5 mm. or less, so that much of the myoglobin can be readily reduced.*

Use has been made by Millikan † of the presence of myoglobin in the muscle fibres for studying the utilisation of oxygen by living muscles. Light is passed through the living soleus muscle of the cat, and falls on to a differential photo-cell colorimeter, by means of which a continuous record of the degree of oxygenation of the myoglobin is made on photographic paper. When the muscle was tetanised the myoglobin showed reduction in 0·2 sec., and had lost up to 40 per cent. of its oxygen in the first second, even when the blood supply was intact. On relaxation the oxygen was restored. These facts are of importance in emphasising the speed with which reactions occur in the mammalian tissues with intact circulation.

* CAMPBELL. *J. Physiol.*, 1925, **60**, 20.
† MILLIKAN, *Proc. R. S.*, 1937, **123B**, 218.

The enormous avidity of the tissues for oxygen is shown by the following experiment (Ehrlich).* If a saturated solution of methylene blue be injected into the circulation of a living animal and the animal be killed ten minutes later, it is found, on first opening the body, that most of the organs present their natural colour, although the blood is of a dark blue colour. On exposure to the atmosphere, all the organs acquire a vivid blue colour. This phenomenon is due to an intense reducing activity of the tissues, whereby hydrogen is added to the methylene blue with the production of a colourless reduction product, which on re-exposure to oxygen is oxidised, by loss of hydrogen, into the blue compound. It is shown by surviving minced tissues as well as *in vivo*. In this reaction the tissue obtains oxygen, for carrying out its

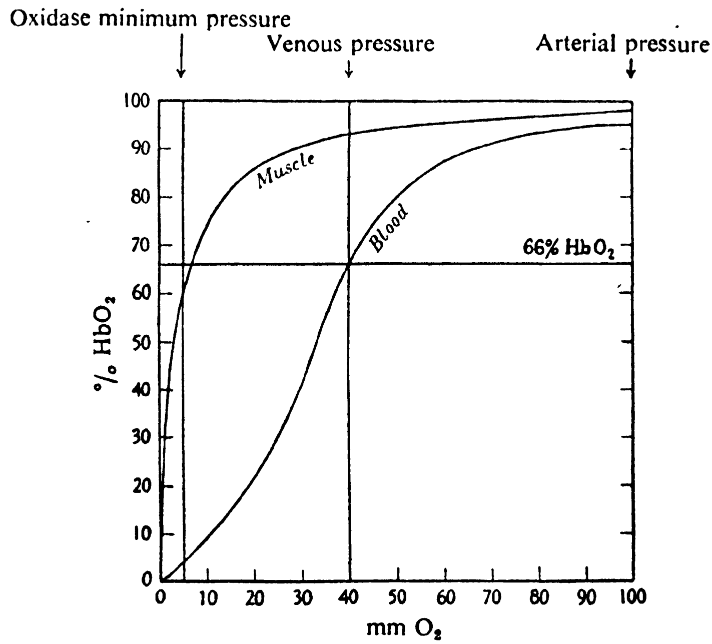

FIG. 488. Dissociation curves of muscle hæmoglobin (myoglobin) and of blood, showing relation to minimum oxygen pressure required by the tissue oxidases. (After R. HILL, *Proc. R. S*, 1936, 120B, 472.)

oxidative processes, by the decomposition of water, the oxygen from the water being transferred to an oxidisable substance (oxygen acceptor), provided there be present a substance, such as the methylene blue (hydrogen acceptor), which can take up the active hydrogen (*v.* p. 104).

TISSUE RESPIRATION. All the tissues use oxygen and form carbon dioxide, the amounts varying with the activity of the tissues at the time of observation. The gaseous exchanges of an organ can be measured by determining the rate of blood flow through it, and by finding how much oxygen is abstracted from, and how much CO_2 is added to, the blood in its passage through the organ. The heart, brain and liver are among the most actively respiring organs, but during muscular work the muscles, which use but little oxygen when at rest, consume very large amounts.

* EHRLICH. "Das Sauerstoff-Bedürfniss des Organisms" (Berlin: Hirschwald, 1885); *Dtsch. med. Woch.*, 1886, No. 4. For a modern discussion regarding the physico-chemical significance of the reduction of dyestuffs, *v.* MANSFELD CLARK *et al.*: *Hyg. Lab. Bull. U.S. Publ. Health Service*, 1928, 151; *Chem. Reviews*, 1925, **2**, 127.

The circulation or presence of blood is not essential to the carrying out of tissue respiration, provided that oxygen is adequately supplied in some other way. Thus, as described for muscle, on p. 146, surviving slices of tissue, or minced tissues, still respire when placed in pure oxygen, and may even do so at rates comparable with those seen in their normal surroundings. The more the tissue structure is destroyed, however, the smaller the oxygen utilisation becomes, and when finally the tissues are ground to a paste, their respiratory activity may be almost entirely abolished. Cellular structure is therefore essential, which means that there are definite apparatus involved in the various stages of the chemical reactions which intervene in the course of the oxidative formation of carbon dioxide.

The study of the respiration of tissue slices by means of various forms of microrespirometers is now much in vogue. For this purpose the slices must be thin enough to ensure adequate oxygen supply, and must be suspended in some suitable isotonic fluid. To this fluid various substances of metabolic importance can be added, and the oxygen uptake followed. The results are expressed as so-called Q_{O_2} values, *i.e.* μl, of oxygen consumed per mg. dry weight of tissue per hour. For instance, the following figures show the combustion of glucose and of pyruvic acid by rat-brain cortex in a phosphate buffer medium (Jowett and Quastel *).

Addition to medium	Q_{O_2}
None	$2{\cdot}89 \pm 0{\cdot}18$
Glucose, 0·01 m. . . .	$12{\cdot}20 \pm 0{\cdot}33$
Pyruvate, 0·02 m. . . .	$11{\cdot}10 \pm 0{\cdot}65$

We have already seen in Chapter IX that oxidation in the tissues is a result of complicated chains of reactions, many of which involve removal of hydrogen. The hydrogen is passed on from more easily reducible to less easily reducible substances, until finally the hydrogen is united with oxygen. In Chapter X we have seen something of the type of changes involved in the metabolism of striated muscle, and we may take it that similar changes occur in other tissues.

There are probably several alternative paths by which oxidative reactions take place. Some of these have already been referred to in Chapter IX, but probably one of the most important is that in which cytochrome is involved. According to Keilin, cytochromes act as intermediate carriers of oxygen, in collaboration with tissue dehydrogenases and cytochrome oxidase. First a tissue metabolite is oxidised by hydrogen removal by a dehydrogenase, the hydrogen being taken up by cytochrome, which is reduced. The reduced cytochrome is then oxidised by free oxygen in presence of the cytochrome oxidase. Cyanides check the action of the oxidase, and so stop respiration due to the cytochrome cycle. The importance of the cycle is indicated by the fact that the greater part of the oxygen usage of all tissues is stopped by quite small traces of cyanides.

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* JOWETT and QUASTEL, 1937, **31**, 565.

CHAPTER XL

THE REGULATION OF THE RESPIRATORY MOVEMENTS

Each movement of inspiration involves the co-ordinated activity of a large number of muscles, and the extent to which they contract will determine the depth of the inspiration. Similarly, if expiration is to take place, they must simultaneously cease to act. The rhythm and extent of the alternate contractions and relaxations of the respiratory muscles are so regulated that the total ventilation is sufficient to keep the composition of the gas in the alveoli at a practically constant level.

Each act of inspiration involves a discharge along a number of nerves, *e.g.* the facial to the muscles moving the alæ nasi, the vagus to the muscles of the larynx, the branches of the cervical and brachial nerves to the muscles of the neck, the phrenic nerves to the diaphragm, and the thoracic nerves to the intercostal muscles. These nerves are derived from motor nerve cells, situated at various levels in the medulla and spinal cord. In each act of inspiration or expiration the activities of all these groups of cells must be correlated.

RESPIRATORY CENTRES

The co-ordination is effected by the subjection of these motor nuclei to the action of specialised portions of the central nervous system, which act as receiving centres for afferent impressions from the lungs and various other parts of the body, and which are also sensitive to changes in the composition of the blood circulating through their vessels. These dominant parts are called *respiratory centres.* If the spinal cord be cut across below the seventh cervical nerve roots, the respiratory action of the intercostal and abdominal muscles ceases permanently, although respiration is still continued by the diaphragm and the other muscles supplied by nerves leaving the central nervous system above the point of section. It has been known since Galen that division of the cord at the first or second cervical nerves abolishes the action of the diaphragm as well, though ineffectual movements of the muscles supplied by the facial, vagus, and spinal accessory nerves continue. A section of the brain stem through the mid-brain leaves the respiratory movements unaltered. We must conclude from these experiments that the respiratory moto-neurons of the cord are subject to control by impulses originating in the hind brain, and transmitted therefrom down the spinal cord.

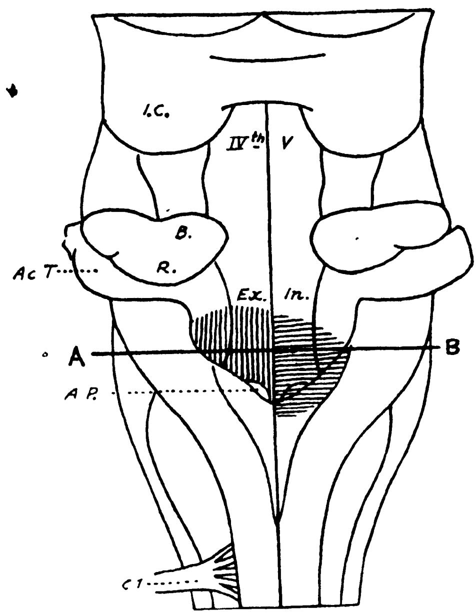
Many experiments have been made with the idea of locating the position of the medullary respiratory centre more accurately. Legallois and Flourens * described it as near the apex of the *calamus scriptorius.* The centre is bilateral, each centre presiding more especially over the muscles of the same side of the body, so that longitudinal section at the middle line does not destroy the respiratory movements †; it lies in the reticular formation, in close connection with the central terminations of the vagus nerves.

* Historical account *v.* Langendorff, in NAGEL'S *Hdbch. d. Physiol. d. Menschen,* 1909, IV 334.

† PORTER. *J. Physiol.,* 1895, 17, 455.

Accurate delimitation of the respiratory centre has been made in the cat.* It is probably similar in position in man. By electrical stimulation of known regions of the brain stem respiratory responses were obtained from two neighbouring neurone pools, one giving inspiration and one expiration, so showing clearly for the first time that there is both an *inspiratory and an expiratory centre.*

The *inspiratory centre* is in the ventral reticular formation immediately



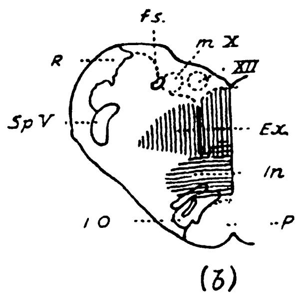


FIG. 489. Diagram showing the positions of the medullary respiratory centres in the cat.

(*a*) Dorsal view of brain stem after removal of cerebellum. Shaded areas show projection of respiratory centres on to floor of IVth ventricle Vertical shading Ex on left, area which on stimulation causes maximal expiration; horizontal shading In, on right, area which on stimulation causes maximal inspiration.

(*b*) Section along line A.B. of (*a*) showing position of areas projected, and showing that the expiratory centre lies dorsally to the inspiratory centre.

Ac T., acoustic tubercle; A P, area postrema, B, brachium conjunctivum; C.1, first cervical root; R, restiform body; I.O, inferior olive; Sp. V, spinal root of Vth cranial nerve, m.X., motor root of vagus; XII., hypoglossal nucleus; P., pyramid: Ex, In., expiratory and inspiratory centres; f s, fasciculus solitarius.

(Modified from PITTS, MAGOUN and RANSON, *Amer. J. Physiol*, 1939, **126**, 673.)

over the cephalic four-fifths of the inferior olive (Fig. 489). When this region is stimulated by rapid faradic shocks, regular breathing is suspended and a deep inspiration, involving both thorax and diaphragm, occurs, and the breath is held in that position as long as the stimulus is kept up (Fig. 490, A).

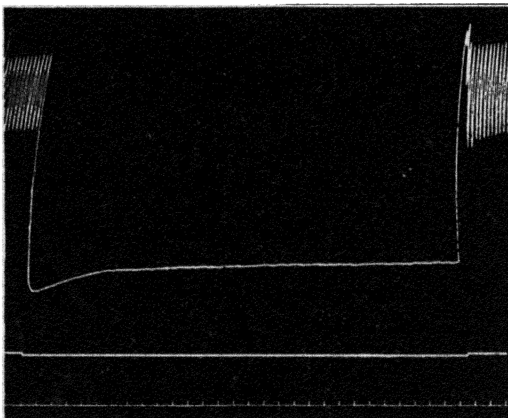
The *expiratory centre* lies slightly cephalic and dorsal to the inspiratory centre. When it is stimulated a full expiratory standstill occurs (relaxation of diaphragm and other inspiratory muscles, and often also a contraction of

* PITTS, MAGOUN and RANSON. *Amer. J. Physiol.*, **1939**, **126**, **673**.

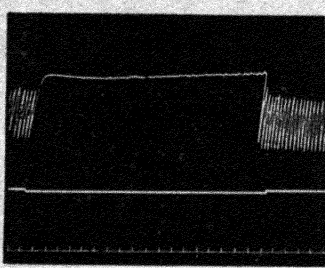
expiratory muscles) and this expiratory position is kept up (within limits) for as long as the stimulation is maintained (Fig. 490B). On prolonged stimulation (over about 3 mins.), however, inspiration breaks through.

The inspiratory centre is the dominant one if both are equally stimulated together: by alternate stimulation of the two centres regular respirations of any desired depth or frequency can be obtained.

The neurones of the two centres are freely connected (Fig. 493), so that irradiation over the whole of a centre follows if only a small part of it is excited;



A



B

FIG. 490. Spirometer tracings of respiratory reactions to stimulation of respiratory centres in the medulla of the cat. Same strength of stimulation used in both records, viz. 8 volts at 240 stimuli per second. Duration of stimulation shown by signal line. Time, 6 second intervals. Inspiration down, expiration up. A. Maximal inspiratory standstill on stimulation of inspiratory centre. B. Maximal expiratory standstill on stimulation of expiratory centre. (PITTS, MAGOUN and RANSON, *Amer. J. Physiol*, 1939, **126**, 673.)

moreover, the other centre of opposite function is also affected, but in the opposite direction. Thus a stimulus applied to the inspiratory centre spreads over the whole of that centre causing excitation, and also spreads to the expiratory centre, causing it to be inhibited. This reciprocal action between centres recalls the "half-centre" functions of limb locomotor centres advanced by Graham Brown (p. 279).

Like all complex co-ordinated movements, the act of respiration, though capable of being carried out through the control of neurone pools such as the medullary centres in question, is subject to various refinements of adjustment which can be brought into play through the intervention of superior centres.* These centres have sometimes been

* V. E. HENDERSON and SWEET. *Amer. J. Physiol.*, 1929, **91**, 94.

regarded as having exclusively respiratory functions and so have often been grouped with the medullary centre as representing specialised departments of a central respiratory control mechanism.

According to Lumsden, for example,* the central respiratory mechanism consists of three parts, viz.:

(a) A lower centre below the striæ acusticæ, which, when working alone, causes gasping respirations ('the gasping centre').

(b) An intermediate centre at the level of the striæ acusticæ, which when working together with the lower centre sends out impulses causing a series of prolonged inspirations ('the apneustic centre').

(c) A dominant part in the upper pons, which inhibits the exaggerated activity of the apneustic centre and so produces normal respiration ('the pneumotaxic centre').

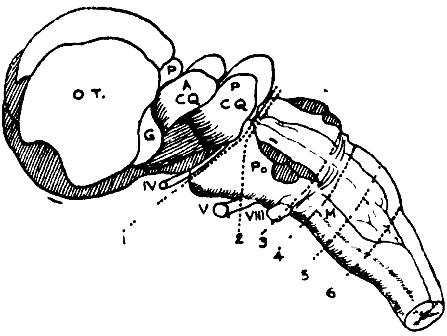


FIG. 491. Diagram of Brain Stem of Cat, showing Levels at which cross section alters the Type of Respiratory Movement. (LUMSDEN.)

Section 1 leaves respiration unaltered.

Section between 2 and 3, causes development of inspiratory type of respiration (apneusis), if vagi are also cut.

Section 4 causes gasping respiration.

Section 6 abolishes respiratory movements.

The relative positions of these centres are shown in Fig. 491, and their effects on respiration in Fig. 492. As will be shown later, these conceptions now need modification. The 'apneustic centre' is probably the inspiratory centre, apneusis being due to inertia of the expiratory centre when this is cut off from afferent impulses from the pneumotaxic centre and from the vagus. Gasping is probably an expression of a deteriorated, though, still dominant, inspiratory centre. Barcroft,† however, considers the gasp to be the basic and most primitive act, which is converted into normal breathing by the effect of various afferent impulses.

From the centres on each side, the efferent impulses to the motor nuclei of the respiratory muscles pass down in the ventral and ventro-lateral columns of the cord. Hemisection of the upper cervical cord, *e.g.* on the right side, causes cessation of the contractions of the diaphragm on the same side. There must, however, be commissural fibres joining the motor nuclei on the two sides. If the right phrenic nerve be divided, after hemisection on the left side, the left half of the diaphragm at once commences to contract rhythmically with each respiration.‡ It is evident that the cessation of respiration after section of the cord is due to the fact that the spinal motor centres cannot act without higher control and is not due to a

* LUMSDEN *J. Physiol.*, 1923, **57**, 153, 354; **58**, 81, 111.

† BARCROFT, J. Features in the Architecture of Physiological Function. (Camb. Univ. Press.)

‡ PORTER. *J. Physiol.*, 1895, **17**, 455.

condition of shock of the lower spinal centres, since it is possible for impulses to pass down the cord and to cross over to the contra-lateral diaphragm nucleus *immediately* after hemisection of the cord on the side of the nucleus.

CORTICAL CONTROL. It is common knowledge that respiratory movements can voluntarily be controlled in any direction for a time, and in

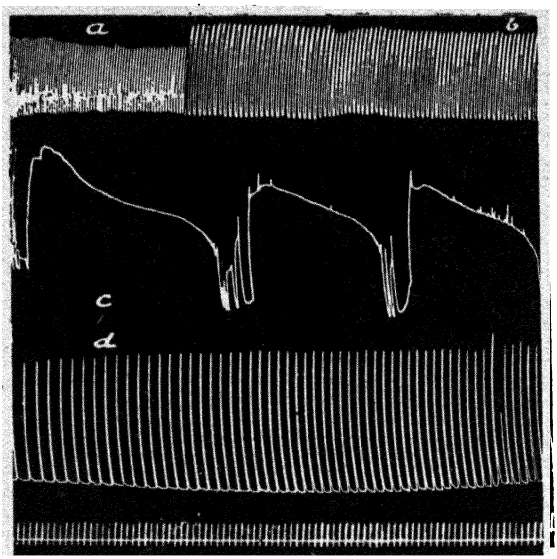


FIG. 492. Tracings of various Types of Respiration in the Cat. (LUMSDEN.) (*a*) Normal. (*b*) After vagotomy. (*c*) Prolonged inspiratory tonus (*apneusis*) after section 2, Fig. 491, and vagotomy. (*d*) Gasping respiration after section 4. Time tracing = 5 secs. Inspiration upwards.

accordance with this, certain parts of the cerebral cortex can be shown to affect the breathing when stimulated. Certain parts of area 6 of the cortex in cats, dogs and monkeys when stimulated cause acceleration, while the excitation of other areas gives inhibition of breathing. These areas have corresponding cytoarchitecture in all these species.*

The Automaticity of the Respiratory Centres

The respiratory centres are often described as automatic. By this we mean that, although they can be affected reflexly, or by chemical changes in the blood, their rhythmic activity is primarily dependent on the metabolism of their own cells.† It is true that the centres continue to function rhythmically after almost complete isolation, *e.g.* after afferent impulses have been cut off from them by section of the brain stem at the corpora quadrigemina, of the cord at the eleventh thoracic segment, of the dorsal roots of all the spinal nerves above that, and of both phrenics, the vagi, fifth, seventh and eighth cranial nerves.‡ Taking the brain stem centres as a whole, therefore, it does seem that they can act independently of the receipt of afferent impulses. But if we separate off the pontine pneumotaxic centre from the medullary centres by a section across the lower pons, after section of the vagi,

* SMITH. *J. Neurophysiol.*, 1938, I., 55.
† GESSELL and MOYER *Q. J. Exp. Physiol.*, 1935, **25**, 13.
‡ STELLA. *J. Physiol.*, 1938, **93**, 10.

rhythmic respiration is at once replaced by apneusis. Either the vagus, or the pneumotaxic centre, must therefore be left in connection with the medullary centres if these are to continue to function rhythmically. Otherwise the inspiratory centre enters into sustained activity, causing apneusis.

The vagus is connected chiefly with the expiratory centre, and Fig. 493

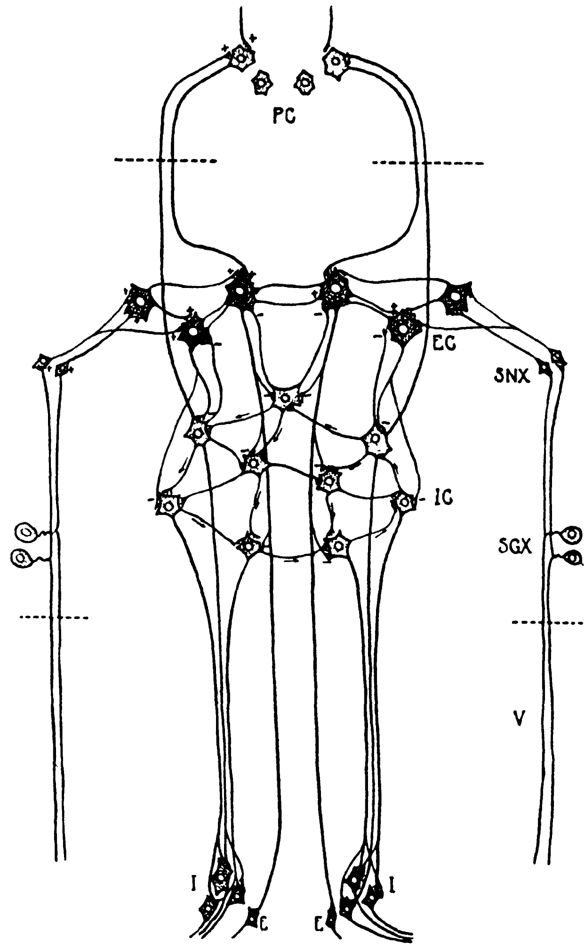


FIG. 493. Diagrammatic representation of possible linkages which would account for rhythmic respiration and apneusis. E, Expiratory, and I, inspiratory motoneurons in cord; E.C. and I.C., expiratory and inspiratory centres; P.C. pneumotaxic centre in pons; V, vagus; SGX, sensory ganglion of vagus; SNX, sensory nucleus of vagus. (PITTS, MAGOUN and RANSON, *Amer. J. Physiol.*, 1939, **126**, 689.)

suggests the kind of linkages in the central nervous system which would account for the phenomena of rhythmic breathing and apneusis. The pneumotaxic centre is in the tegmentum of the extreme upper part of the pons, and is connected with the medullary centres by a pathway ventral and slightly medial to the descending root of the fifth cranial nerve.*

The respiratory centres as a whole tend to respond to all stimuli, continuous or rhythmic, by means of rhythmic discharges. This means that

* PITTS, MAGOUN and RANSON. *Amer. J. Physiol.*, 1939, **127**, 654.

the inspiratory and expiratory centres tend to function alternately, as also do many other opposed motor centres, such as those concerned with flexion and extension movements of the limbs.

If we increase the activity of the centre, and therefore its gaseous interchanges, by warming the blood in the carotid arteries, there may be a considerable quickening of respiration unaccompanied by any deepening, a condition which is spoken of as *tachypnœa*. On the other hand, we may slow the respiratory movements by cooling the floor of the fourth ventricle.

THE CHEMICAL REGULATION OF THE RESPIRATORY MOVEMENTS

Asphyxia. If the proper aeration of the blood be interfered with in any way in an intact animal, the respiratory movements increase in depth and frequency, and if the disturbing factor be not removed, the animal dies of 'asphyxia.' The asphyxia may be general, as by occlusion of the trachea, by pneumothorax, &c., or local, as by ligature of the blood vessels supplying the brain.

The phenomena of asphyxia may be divided into three stages:

(1) In the first stage, that of *hyperpnœa*, the respiratory movements are increased in amplitude and in rhythm. This increase at first affects both inspiratory and expiratory muscles. Then the breathing becomes *dyspnœic*. Gradually, the force of the expiratory movements becomes increased out of all proportion to the inspiratory, and the first stage merges into:

(2) the second, which consists of *expiratory convulsions*, in which almost every muscle of the body may be involved. Just at the end of the first stage consciousness is lost, and almost immediately afterwards there appear a number of phenomena of central excitation extending to almost all the functions of the body. Thus the vasomotor centre is excited, causing universal vascular constriction. There is often also secretion of saliva, inhibition or increase of intestinal movements, constriction of the pupil, and so on.

(3) At the end of the second minute the expiratory convulsions cease almost suddenly, and give way to *slow deep inspirations*. With each inspiratory spasm, the animal stretches itself out and opens its mouth widely, as if gasping for breath. The whole stage is one of exhaustion: the pupils dilate widely, and all reflexes are abolished. The pauses between the inspirations become longer and longer, until, at the end of four or five minutes, the animal takes its last breath.

In the production of the phenomena of asphyxia, two factors are present, namely, an increase of carbon dioxide and a diminution of oxygen in the blood bathing the centre, or in the centres themselves. The question arises, to what extent is each of these responsible for the events which characterise asphyxia? The answer is that both factors contribute, though in different ways, just as we saw that both factors are involved in causing the changes in the circulation in asphyxia (p. 680).

The Normal Chemical Stimulus. At various times the stimulus for respiration has been ascribed, either to the increased tension of carbon dioxide, or to the diminished tension of oxygen in the arterial blood. Increase of this stimulus, it was believed, augmented the activity of the centre and caused hyperpnœa, reduction lowered its activity, so that breathing was suspended—a condition called *apnœa*. Haldane and his pupils believed that the respiratory centre could itself be excited either by excess of carbon

dioxide or by lack of oxygen, but that its sensitivity to carbon dioxide was by far the more important factor in the determination of the increased respiratory movements in asphyxia, and was the only chemical variable in the blood which could be regarded as playing a large part in the regulation of the respiratory movements under normal conditions. This factor is

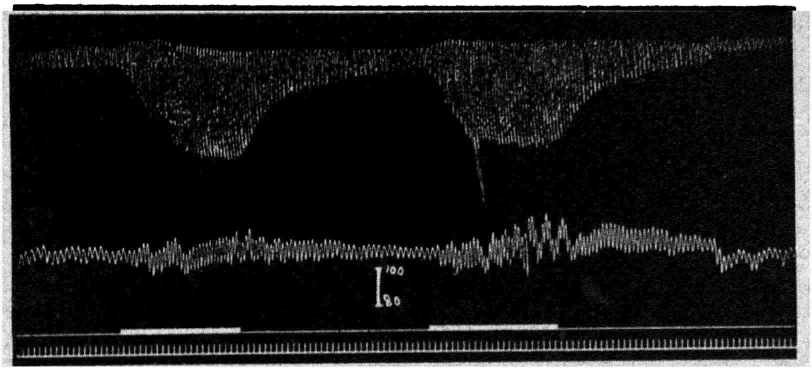


Fig. 494. Effect of CO_2 on Respiratory Movements of Rabbit.
(Scott. *J. Physiol.*, 1908, 37, 301.)

Upper line, tracing of diaphragm slip (Head's method) Lower tracing, carotid pressure. During the first period indicated on the signal line the animal breathed 9·6 per cent. CO_2 in air, and during the second period 10 per cent. CO_2 with 33 per cent. oxygen. Time tracing = 2 secs. Scale = mm. Hg blood pressure.

well brought out if we investigate in man the effect on the respiratory movements of altering the tensions of the two gases in the air breathed.* If, by this means, we alter the tension of the two gases in the alveolar air, we may assume that the tensions of the gases in the arterial blood are similarly changed.

Carbon Dioxide. Even a slight increase in the percentage of carbon dioxide in the air causes an increase, first in the depth and later on in the rhythm, of respiration (Fig. 494). This is shown in the following Table (Haldane and Priestley), which represents the average ventilation effected by the respirations when the subject was breathing either normal air or air

CO_2 p.c. in inspired air	Mean CO_2 p.c. in alveolar air	Relative rates of alveolar ventilation
0·03	5·71	100
1·74	5·65	143
3·98	6·03	277
5·28	6·55	447

containing varying percentages of carbon dioxide. For instance, a rise of carbon dioxide *in the atmosphere* to 1·74 per cent. increases the total alveolar ventilation by 43 per cent.

The second column of figures in this Table shows that, in spite of the very large variations in the air breathed, the alveolar content of CO_2 remained practically constant until the CO_2 in the atmosphere was increased to such an extent that the processes of compensation were no longer efficient.

* Haldane and Priestley. *J. Physiol.*, 1905, 32, 225.

We must conclude, therefore, that the pulmonary ventilation is so arranged as to react to the slightest increase of CO_2 tension in the blood, any increase in this giving at once a compensatory increase in ventilation, so that the alveolar CO_2 pressure may be maintained almost constant.

That it is the *partial pressure*, and not the percentage amount of CO_2 in the alveolar air and therefore in the blood, which is really the determining factor, is shown by a comparison of the composition of the alveolar airs of subjects under different atmospheric pressures. In calculating the partial pressure from the percentage composition and barometric pressure, deduction of 47 mm. is made from the latter to allow for the tension of aqueous vapour at body temperature. Thus at sea level (barometer = 760 mm.), the alveolar CO_2 content of a subject was 5·3 per cent., which represented an alveolar CO_2 pressure of 37·8 mm. Hg. When the subject was placed in an air-chamber compressed to a pressure of 4640 mm., the mean percentage of CO_2 in the alveolar air was 0·9, corresponding, however, to a *tension* of 41·3 mm. Hg. Thus, the pressure of CO_2 in alveolar air remains practically constant with widely varying limits of atmospheric pressure as well as with very different percentages of CO_2 in the inspired air, showing that the reactions of the organism are directed so as to maintain, by alterations in the ventilation, a constant *tension* of this gas in the alveoli, and therefore in the arterial blood (Fig. 495).

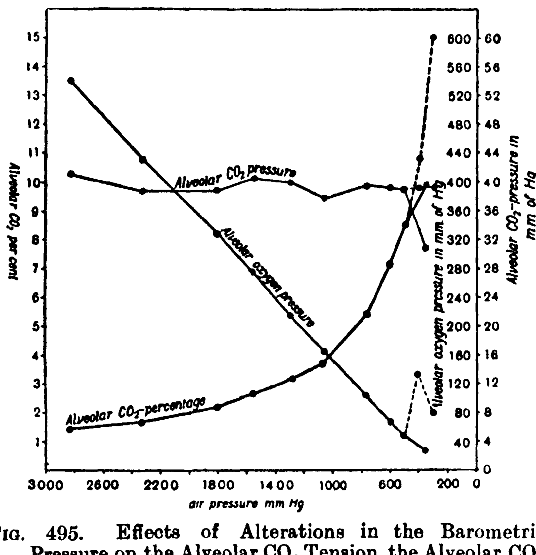


FIG. 495. Effects of Alterations in the Barometric Pressure on the Alveolar CO_2 Tension, the Alveolar CO_2 Percentage and on the Alveolar O_2 Tension. Note that the excitant effects of O_2 lack are not seen until the atmospheric pressure falls below 500 mm Hg. (BOYCOTT and HALDANE, *J. Physiol.*, 1908, **37**, 355.)

Actually it is the ratio of CO_2 to $NaHCO_3$ in the blood (*i.e.* the pH of the blood) which is kept constant. Hence if the bicarbonate of the blood is altered, the pCO_2 of the blood is adjusted by an alteration of the breathing. Just after a meal, for instance, when the HCl of the gastric juice is being separated off from the blood, there remains an excess of base, in the form of bicarbonate in the blood. This leads to a reduction of the breathing so that the alveolar CO_2 shows a slight rise.* A similar result follows if bicarbonate is taken by the mouth † and the converse result if acid or ammonium chloride is taken.‡ In the latter case the ammonia is converted into urea leaving the HCl behind in the blood. The alveolar CO_2 shows a definite fall; it does this under any circumstances, *e.g.* in diabetes, where there is an excess of acid in the blood. In all cases the effect of the response is to regulate the output of CO_2 so that the ratio of $\frac{CO_2}{NaHCO_3}$ remains constant.

While these facts and deductions are incontrovertible, they do not justify the conclusion that the effects of altered CO_2 content of the blood are exerted directly upon

* DODDS, *J. Physiol.*, 1921, **54**, 342; DODDS and BENNETT, *J. Physiol.*, 1921, **55**, 381; BENNETT and DODDS, *Brit. J. Exp. Path.*, 1921, **2**, 58.

† DAVIES, HALDANE and KENNAWAY, *J. Physiol.*, 1920, **54**, 32.

‡ J. B. S. HALDANE. *J. Physiol.*, 1921, **55**, 265.

the respiratory centres. The effect might be upon some peripheral structure from which the respiratory centre is affected secondarily by reflex action. Experiments on man do not permit of an answer to this objection, which, as will be shown later, is in part justified. We may state here, however, that even when, in animals, all likely afferent nerves are severed, respiration is still augmented by a rise in the CO_2 content of the blood supplied to the centre, so we may conclude that the centre itself does respond directly to that chemical change in the blood; it responds less than in the intact animal, however, so the effect is partly reflex.

Stimulation by Oxygen Lack. Very different are the phenomena observed on sudden alteration of the partial pressure of oxygen (Figs. 495 and 496). Here, within wide limits, the partial pressure of oxygen in the alveolar air is determined by its pressure in the inspired air. Thus, if we take the above series of observations, with a pressure of 646 mm., the *percentage* of oxygen in the alveolar air was 13·19, corresponding to a *tension* of 78·6 mm. Hg. At an atmospheric pressure of 755 mm. the percentage of

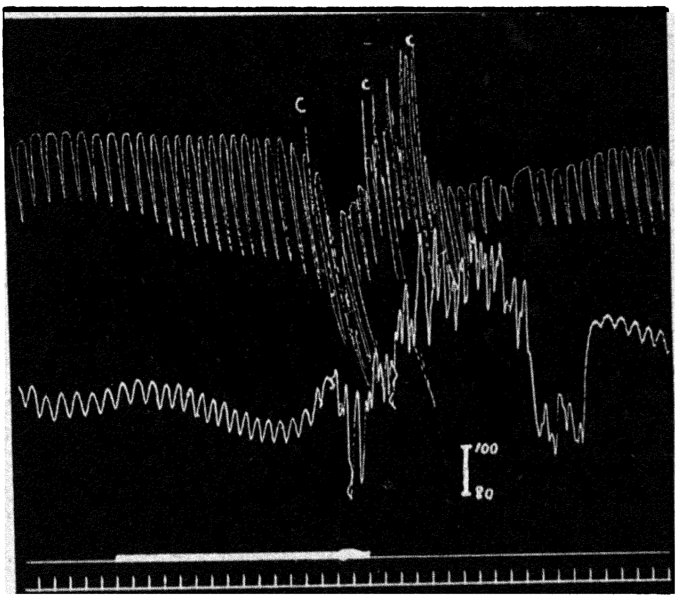


Fig. 496. Effects of Oxygen Lack. (Scott.)

Upper tracing, diaphragm slip; lower tracing, carotid blood pressure. During time indicated by signal, 5 per cent. oxygen in nitrogen was inhaled. c = convulsion.

oxygen in the alveolar air was 13·97, corresponding to a tension of 98·5 mm., which we may take as the normal figure at the sea-level. In air compressed to a pressure of 1261 mm. the percentage of oxygen was 16·79, corresponding to an alveolar oxygen tension of 203 mm. Hg.

Similar results are obtained by altering the percentage of oxygen in the air breathed at constant pressure. The oxygen tension or percentage in the inspired air can be lowered from its normal of 20·95 to 13 per cent., at which the alveolar oxygen is about 8 per cent. (= 57 mm. Hg), without altering the depth or rhythm of respiration, and, in fact, without any change being noticed by the subject of the experiment. With a further reduction of the oxygen content, however, there is increased pulmonary ventilation (Fig. 496), but the diminution in oxygen may be pushed to such an extent that the

patient becomes blue, without any considerable distress being caused. In fact, the subject may lose consciousness suddenly, before he has felt serious discomfort. In all probability such effect as lack of oxygen has upon respiration is not exerted directly upon the respiratory centre, as will be shown later.

The difference in the sensitiveness of the response to increase of carbon dioxide and lack of oxygen respectively, is well shown by an experiment of Haldane's, in which the same person breathed in and out of a bag, in the first place allowing the carbon dioxide produced in respiration to accumulate, and in the second, removing the carbon dioxide by means of soda lime, so that the sole effect of respiration was to produce a continual diminution in the percentage of oxygen. In the first case, when the carbon dioxide was allowed to accumulate, it was found that intolerable hyperpnœa was produced when the content of the bag had reached 5·6 per cent. carbon dioxide and 14·8 per cent. oxygen. When the carbon dioxide was absorbed, no hyperpnœa was produced, and the experiment was stopped as soon as the subject was becoming blue in the face and experienced slight throbbing in the head. The pulse frequency had gone up from 80 to 108. The bag contained no carbon dioxide and only 8·7 per cent. oxygen.

We must conclude that the respiratory apparatus is peculiarly sensitive to variations in the pressure of carbon dioxide, and that these variations are the chief ones which determine the normal depth and rate of the respiratory movements. Although the breathing can be excited by lack of oxygen, this effect is rarely brought into play. Under all ordinary circumstances, an increased need for oxygen is associated with an increased accumulation of carbon dioxide in the body, and the augmentation of respiration, produced by the excitatory effect of a small excess of carbon dioxide tension in the blood, suffices also to provide fully for the increased needs for oxygen.

THE REFLEX REGULATION OF RESPIRATION

Afferent Nerves. Although the sensibility of the respiratory centre to CO_2 is the most important factor in determining the respiratory ventilation, the respiratory centre is modified in a large degree by impulses arriving at the centre along both vagi and both carotid sinus nerves. Through other sensory nerves of the body the respiratory movements can be altered reflexly, but under normal circumstances it is only through these nerves that a continuous stream of impulses passes to the centre, so that every respiratory movement is modified by these impulses.

If both vagi be divided, a marked change in the respiratory rhythm is at once seen. The movements become less frequent, but are increased in amplitude. If, now, the central end of one of the vagi be stimulated with a weak stimulus, a slight inspiratory increase of the movements of respiration results. With a stronger stimulus an expiratory hypertonus results, or a definite expiration. The vagus is connected mainly with the expiratory centre, excitation of which inhibits inspiration and provokes expiration.

This effect may be strikingly brought about by stimulation of the central end of the superior laryngeal nerve, which produces first an inhibition of inspiration and then a forcible expiration. The superior laryngeal nerve supplies sensory fibres to the mucous membrane of the glottis, and we know that the slightest irritation of these fibres—the presence of a crumb or a particle of mucus—causes forcible expiratory spasms, with spasmodic closure, of the glottis, which we term a cough. It must not be imagined that the fibres of the superior laryngeal nerves are concerned in the reflex maintenance of the normal respiratory rhythm. They are cited here merely because the result of their stimulation somewhat resembles that which would be caused

by stimulation of the analogous expiratory fibres which run in the trunk of the vagus from the *lungs* to the respiratory centre.

Since section of both vagi causes slowing of respiration, impulses which exert some influence on the respiratory centres and quicken respiration must travel up the vagi from the lungs. The respiratory movements cause an alternate inflation and deflation of the lungs, and it appears that it is these changes in the volume of the lungs which start the accelerating impulses that travel up the vagus nerves. To test the truth of this hypothesis, it is necessary to see the result, on the respiratory movements, first of distension, and secondly of collapse, of the lungs.

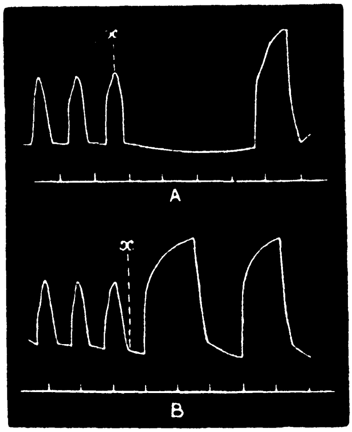


FIG. 497. Effects of Distension and Collapse of Lung. Contraction of diaphragm slip of rabbit. Upward movement = inspiration (contraction). (FOSTER.)

In A, the trachea is closed at x, the height of inspiration; a long pause follows, and finally an inspiration (a very powerful one) sets in.

In B, the trachea is closed at the end of expiration, x; powerful inspirations follow at once.

When it is wished to study the effects of artificial distension or collapse of the lungs we may use the method described by Head.* In the rabbit, a slip of the diaphragm serves as a sample of the diaphragm, the contractions of which may be recorded, uninfluenced by passive movements. Alternatively, in the dog, the movements of the larynx up and down with each respiratory movement can be recorded.

The effects of simply closing the trachea at the end of inspiration or of expiration are shown in Fig. 497, and similar results are obtained by experiments on man.† A still more marked effect is produced if the lungs be artificially inflated. The inflation produces an instantaneous and complete relaxation of the diaphragm (Fig. 498), which by clamping the tracheal tube may be prolonged for several seconds. Forcible deflation of the lungs produces the opposite effect, viz. contraction of the inspiratory muscles.‡

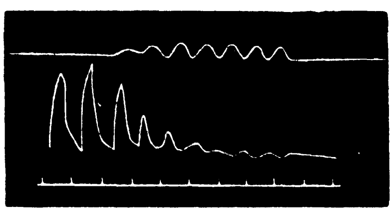


FIG. 498. Positive Ventilation. (HEAD.)

Under the influence of positive ventilation, the inspiratory contractions of the diaphragm become less and less till they disappear completely.

When the action potentials in a single vagus fibre from the lungs are recorded, it is found that it conveys impulses in rapid succession each time the lungs are distended, the impulses being more numerous and in more

* HEAD. *Journ. Physiol.*, 1889, **10**, 1.

† HALDANE and MAVROGORDATO, *J. Physiol.*, 1916, **50**, Proc. xli.; LAMB, "An Introduction to Human Experimental Physiology," p. 183, 1930. (Longmans.)

‡ HEAD. *J. Physiol.*, 1889, **10**, 1; CREED and HERTZ. *Ibid.*, 1933, **78**, 85.

rapid sequence the more powerful the distension.* The lung evidently possesses stretch receptors similar to those of muscle, and like them causing reflexly an inhibition or moderation of the movement that produced the stretch—in this case the act of inspiration (Fig. 499). In collapse of the lung these impulses cease; but on forcible suction fresh impulses ascend the vagus. These are probably not present in normal breathing movements.

It is not known where the receptors which respond to inflation of the lungs are situated, though it seems probable that they are in the walls of the bronchi and bronchial tubes. Fibres of the vagus have been traced to the bronchialis muscle, where they terminate in sensory endings resembling those of the carotid sinus, and presumably, like these, acting as stretch receptors.†

The Hering-Breuer Reflex. These inhibitory and augmentor effects of changes in the volume of the lung must also result from the normal move-

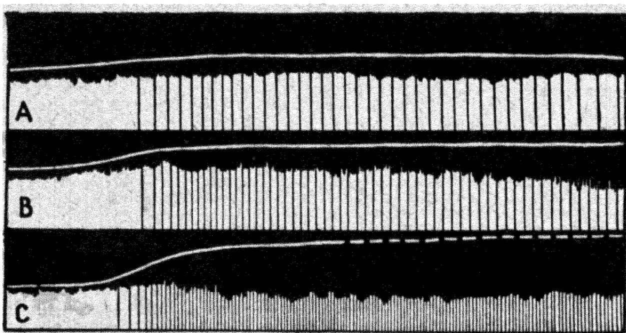


FIG. 499. Action potentials in pulmonary vagus fibres of cat.
A. Spinal Cat. Inflation of 65 c.c. Max. frequency = 80 per sec.
B. Spinal Cat. Inflation of 115 c.c. " " = 120 " "
C. Decerebrate Cat. Natural breathing (one breath).
The light line shows degree of inflation of lung. (ADRIAN. *J. Physiol.*, 1933, **79**, 332.)

ments of respiration. Let us consider, for instance, what will happen if the influence of the two vagi could be suddenly restored after these nerves have been divided. (This experiment can in fact be realised if the functional division of the vagi be effected by cooling.) The animal would be breathing slowly and deeply. If, at the beginning of an inspiration, the vagi became functional, the expansion of the lungs caused by the inspiratory movement would send impulses up the vagus to the expiratory centre, which would arrest the movement of inspiration. The movement of expiration would then begin, but the collapse of the lungs thereby produced would stop the impulses up the vagi, and so allow the inspiratory centre to cause an inspiratory movement. Both inspiration and expiration would therefore be shortened, and the successive movements would follow one another at a shorter interval than if the vagi were not functional. This reflex is called the Hering-Breuer reflex.‡ It is abolished when both vagi are divided (Fig. 500). By its operation the *rhythm* of the respiratory centre must be determined reflexly through the agency of the vagi, while the chief factor in determining

* ADRIAN. *J. Physiol.*, 1933, **79**, 332.
† GAYLOR. *Brain*, 1934, **57**, 143
‡ HERING and BREUER. *Sitz. d. Wien. Akad. Math.-Nat. Cl.* (2), 1868, **57**, 672; **58**, 909.

the total pulmonary *ventilation* is, as we have seen, the carbon dioxide tension of the blood.

Though the effect of double vagotomy is to produce slowing of the respiration, it is necessary to answer the objection that this may not be due to the section of the pulmonary fibres in the vagus, but to the division of fibres running in the trunk of the vagus from some other source. We know that other afferent fibres which would also be divided can influence respiration, *e.g.* the fibres of the "depressor" nerve, from the cardio-aortic area, and perhaps the fibres of the recurrent laryngeal nerve.* These objections have been answered by the experiment of dividing these nerves, while leaving the pulmonary branches of the vagus intact, a procedure which leaves the respiratory rate unchanged, and then by the converse experiment of sectioning only the pulmonary branches of the vagus, which produces the same slowing as division of the entire cervical vagus,† and, like it, abolishes the Hering-Breuer reflex (Fig. 500).

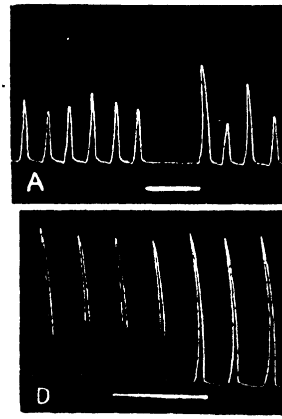


FIG. 500. Respiratory movements of larynx of chloralosed dog. A. The left vagus has been divided in the neck. Inflation of lungs during white line arrests respiration. D. The pulmonary branches of the right vagus have now been divided. The Hering-Breuer reflex is abolished. (ANREP and SAMAAN. *J Physiol.*, 1932, 77, 1.)

Although extreme collapse of the lung may cause afferent impulses to pass up the vagi, it seems certain that these are not engendered by the moderate degree of collapse accompanying normal expiration. When such impulses are produced by the unusual stimulus of strong suction, they seem able to excite the opposite reflex to that produced by inflation, viz. an inspiratory movement. The production of inspiration by weak stimulation of the central end of the vagus is further evidence that such a reflex is feasible though it does not justify the assumption that it is involved in the production of normal inspiratory movements.

Collaboration of Nervous and Chemical Control. After division of both vagi, the total pulmonary ventilation does not, as a rule, undergo any marked change, and the aeration of the blood is carried out as well as in the intact animal. The effect of the impulses travelling up the vagi, by reining in the activity of the centre, is to economise its energy and the energy of the respiratory muscles. The result of the vagal impulses will therefore be to increase the delicacy of the respiratory centre and make it more susceptible to slight changes in the carbon dioxide tension of the blood, while maintaining a sufficient margin of energy to meet the increased needs thrown on the respiratory mechanism by augmented metabolism, such as occurs in violent muscular exercise. The importance of the vagus action for the organism is shown if we put an increased strain on the respiratory mechanism, as, for instance, by increasing the carbon dioxide in the air breathed.‡ In the intact animal, this procedure leads first to increased depth, and later to increased frequency of respiration, the total ventilation being thereby augmented to such an extent as to keep the alveolar tension of carbon dioxide almost constant. If the same percentage of carbon dioxide be administered to a chloralised rabbit after section of both vagi, the effect is

* SHARPEY SCHAFER. *Q. J. Exp. Physiol.*, 1919, **12**, 231.
† ANREP and SAMAAN. *J. Physiol.*, 1932, **77**, 1.
‡ SCOTT. *J. Physiol.*, 1908, **37**, 301.

deepening of respiration, but not quickening (Fig. 501). Each inspiratory movement, however, is already considerable, so that the margin by which increase of pulmonary ventilation is possible, by increase of depth of respira-

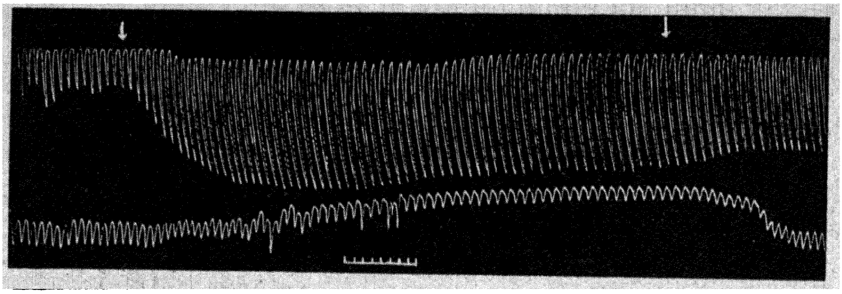


FIG. 501. Effect of 10·6 per cent. CO_2 in a Mixture containing 23 3 per cent. O_2 on a Rabbit with both Vagi divided. (F. H. SCOTT, *J. Physiol.*, 1908, 37, 301.)

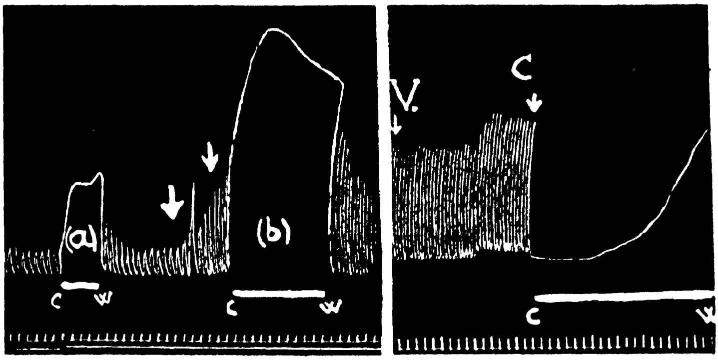
The gas was administered between the arrows. Zero line of blood pressure is 32 mm. below bottom of tracing. Compare this figure with Fig. 494, p 789.

tion alone, is not so great as in a normal animal. Moreover, since no quickening of respiration takes place, the increased ventilation rapidly becomes inadequate for the maintenance of the normal alveolar carbon dioxide tension. In the following Table the total amounts of pulmonary ventilation, obtained on administration of mixtures containing carbon dioxide to a rabbit before and after section of the vagi, are compared.

	Respirations per minute	Vol. of each respiration	Total ventilation per minute
		c c	
Respiration with air . . .	72	19	1368
„ 4·2 per cent. CO_2 .	96	25	2400
„ 8·6 per cent. CO_2 .	97	29	2813
VAGI DIVIDED			
Respiration with air . . .	45	29	1305
„ 4·2 per cent. CO_2 .	45	34	1530
„ 8·6 per cent. CO_2 .	42	38	1596

THE VAGUS AND APNEUSIS. We have seen that the respiratory centre (*i.e.* the inspiratory and expiratory centres) cannot function rhythmically when isolated from both the pneumotaxic centre and the vagus. Hence, if the influence of the pneumotaxic centre is removed by section across the upper pons, suspension of the action of the vagi, as by local cooling, produces a prolonged holding of the breath in the position of inspiration, a state called apneusis. This is due to a condition of *tonic* activity of the inspiratory centre and is caused by the presence of carbon dioxide in the blood reaching the centre. If the carbon dioxide tension in the blood is raised, as by breathing air containing CO_2, the apneustic contraction of the inspiratory muscles is increased, while if CO_2 in the blood is lowered by excessive artificial respiration, the apneusis remains in abeyance until such time as the CO_2 of the blood

has mounted up to a level adequate to excite the centre (Fig. 502). When the action of the vagi is restored again by re-warming the vagi, apneusis ceases because the expiratory centre is now stimulated by the afferent



A B

FIG. 502. Illustrating the effect of CO_2 content of the blood on apneusis. Cat decerebrated by section through the upper pons. Between *c* and *w*, vagi were blocked by local cooling. Between arrows in A, inhalation of air containing 8 per cent. CO_2. At V, in B, excessive artificial ventilation of the lungs with air. Time, 4 secs. (STELLA, *Journ. Physiol.*, 1938, **93**, 263.)

impulses along the vagi from the stretched lung, and so rhythmic breathing is restored.

Chemical Respiratory Effects on the Carotid Body

An important means by which the activity of the respiratory centre may be influenced was discovered in 1927 by C. Heymans. This is the influence of the chemical composition of the blood upon nerve endings in the aorta and carotid body, which have already been shown to be important regions in connection with the regulation of arterial blood pressure and heart rate; they, and especially the carotid body, must now be regarded as also of importance in the regulation of the respiratory movements.

That alteration in the composition of the blood in the aorta caused respiratory reflexes was shown by the fact that if the head of a dog was separately perfused, changes of blood composition in the trunk still caused respiratory changes in the larynx movements, provided the vagi were intact.*

It has been shown by Heymans and his collaborators,† that when the carotid sinus region, with its nerve supply intact, is isolated from the circulation by appropriate ligatures, and perfused with blood which never enters the general circulation, and so never reaches the respiratory centre at all, definite respiratory reflexes are produced when the composition of that blood is changed; these are of the same nature as would have resulted had the blood which was passed through the carotid sinus region been also sent to the respiratory centre (Fig. 503).

Thus, perfusion of the sinus with blood containing an excess of CO_2 or a deficit of oxygen, or with asphyxial blood, causes hyperpnœa, while perfusion

* HEYMANS and HEYMANS. *Arch. int. pharmac. therap.*, 1927, **33**, 273.

† HEYMANS and BOUCKAERT, *J. Physiol.*, 1930, **69**, Proc. xiii.; BOUCKAERT, DAUTREBANDE and HEYMANS, *J. Physiol.*, 1930, **71**, Proc. v.; HEYMANS, BOUCKAERT and DAUTREBANDE, *Pflüger's Arch.*, 1932, **230**, 283.

with blood deficient in CO_2 causes apnœa. Similarly, if the carotid sinus of a dog A is perfused with blood from the carotid artery of a dog B, this blood being returned to B and not allowed to enter the general circulation of A, then over-ventilation of the lungs of B leads to apnœa of A, and

A

P.

B.P.

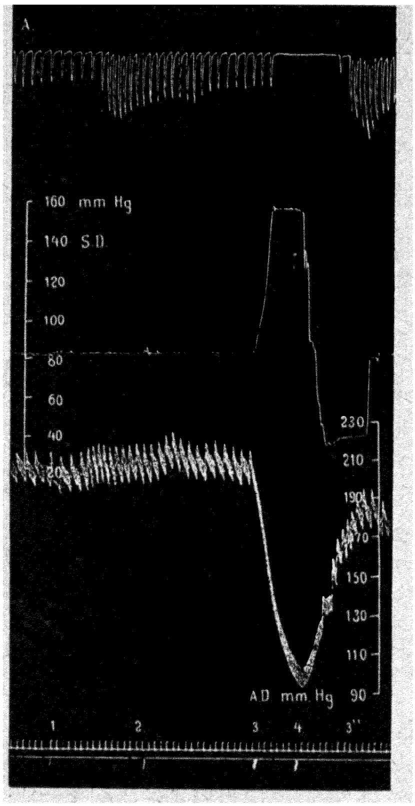


FIG. 503. Effect on respiration of dog of alteration of pressure and of CO_2 content of fluid in the carotid sinuses. The isolated carotid sinus was perfused with a CO_2-Ringer solution from a pump. At the start the fluid contained 11 vols. CO_2 per cent. and had a *p*H of 7·25; at 1 changed to fluid containing 79 vols. p.c. CO_2 and *p*H, also 7·25 (by increasing the $NaHCO_3$); at 2 returned to original fluid; at 3–4 the perfusion pressure was raised; at 4 pressure lowered to 40 mm. Hg. A = respiration. P = perfusion pressure. B.P. = arterial pressure. Time = 3 seconds. (HEYMANS, BOUCKAERT and DAUTREBANDE, *Pflüger's Arch.*, 1932, **230**, 283.)

asphyxia of B to hyperpnœa of A. It is claimed that the carotid body is sensitive to oxygen lack and carbon dioxide excess, and not to the change of *p*H which usually accompanies the latter. When the carotid bodies are denervated these effects of perfusing it are no longer seen. Moreover, the usual effects of lack of oxygen on respiration in the whole animal are not seen if the carotid bodies and aorta are denervated. Under these circumstances oxygen lack rather depresses than excites the respiratory centres.*

The response to oxygen lack is thus mainly due to a reflex from the carotid bodies.

That the chemical effects arise from the carotid body and not from the

* SELLADURAI and WRIGHT. *Q. J. Exp. Phys.*, 1932, **22**, 233.

stretch-receptors of the carotid sinus has been shown by denervating the latter while leaving the innervation of the former intact.* Under these conditions a regular succession of impulses was shown to pass along the carotid body nerve normally, *i.e.* provided the CO_2 pressure in the arterial blood was over 35 mm. Hg. If and when the CO_2 pressure rose the frequency of the impulses was accelerated. Oxygen lack produced a discharge of impulses of a different type.

Adaptation of Breathing. As an example of a normal adaptation, we may take the changes in respiration which occur in an animal as the result of muscular exercise. During activity, the blood, in passing through the lungs, will not be able entirely to get rid of the excess of the carbon dioxide, and will reach the carotid sinus and respiratory centre with a slightly increased CO_2 tension. The carbon dioxide content of the alveolar air will be raised, but the respiratory centre is stimulated both directly and reflexly, and the increased pulmonary ventilation thereby produced prevents further rise in the alveolar carbon dioxide pressure, until a point is reached at which an equilibrium is maintained between the effect of the increased production of carbon dioxide in raising the arterial carbon dioxide tension and that of the increased respiratory activity in lowering it. Under these circumstances it is found that the increased consumption of oxygen in the contracting muscles is compensated; in fact, there is a linear relationship between oxygen consumption and the volume of air breathed, when the exercise is moderate in amount.

When the exercise is violent, so that the animal or man runs into 'oxygen debt,' or when the individual breathes air of low oxygen pressure, the respiratory movements may even be increased to such an extent that the tension of carbon dioxide in the blood falls below normal. Under these circumstances, some other mechanism must be invoked to explain the increased breathing. An important factor, no doubt, is the hydrogen-ion concentration of the arterial blood. During rest or moderate exercise, the reaction of the blood is a function of its CO_2 tension. With violent exercise, two acids, namely carbonic and lactic acids, contribute to the change in hydrogen-ion concentration. If enough lactic acid is produced, the respiratory movements may be augmented to such an extent that the CO_2 tension in the blood and alveolar air falls below normal and yet the hydrogen-ion concentration may be raised, because so much bicarbonate has been neutralised by the lactic acid. Further, there may be deoxygenation of the arterial blood, which causes further respiratory increase by action on the carotid body.

The first phase in the phenomena of asphyxia is conditioned simply by the changes in the carbon dioxide tension. A little later, the gradual exhaustion of oxygen in the blood begins to make itself felt, and the reflexes from the aorta and carotid body, due to this cause, further augment the breathing. During the second stage there is a discharge of energy throughout the whole nervous system, beginning in the bulbar centres and causing a great rise of blood pressure, with slowing of the heart, and extending thence to all the spinal centres with the production of muscular spasms. At this stage too, there is a discharge of impulses causing contraction of the pupil, and a discharge from the whole nervous system. The phenomena of the third stage are due to the influence of oxygen lack upon the nerve centres, accompanied or preceded by exhaustion and dilatation of the heart, the circulation failing before the excitation of the lower centres has entirely come to an end.

* BOGUE and STELLA. *J. Physiol.*, 1935, **83**, 459; SAMAAN and STELLA. *J. Physiol.*, 1935, **85**, 309.

In this third stage, it is impossible, even by the strongest stimuli, to evoke any reflex.

Considerable discussion has taken place as to the exact nature of the stimulus to the respiratory system, when the carbon dioxide pressure of the arterial blood is raised. The breathing is particularly sensitive to this change. For instance, when the CO_2 pressure of the alveolar air is raised by only 2 mm., the breathing is increased about threefold.* Yet there would be a decrease of *p*H of the blood of only 0·012. Can we, then, regard the respiratory apparatus (*i.e.* the centre *plus* the peripheral structures such as carotid body, &c.) as exquisitely sensitive to the H-ion concentration of the blood, and consider this as the real stimulus ? The answer is that we cannot, because, under some conditions, the centre behaves as though it were indifferent to the blood reaction, though sensitive to CO_2 or, sometimes, to the HCO_3' ion. It is admittedly a complex problem, because we cannot easily alter the *p*H of the blood without also altering the CO_2 pressure in the opposite direction. Hence, as a rule, a fall of *p*H does lead to increased pulmonary ventilation.

The real reason for the excitation produced in the respiratory centre by lack of oxygen, which, as we have seen, is a reflex from the carotid body, is even more obscure. Lack of oxygen might reasonably be expected to act, not as a stimulating, but as a paralysing agent, whether on the cells of the respiratory centre or on any peripheral structures. Can we find any common ground on which to explain the action of both oxygen lack and CO_2 excess ?

Gesell, in attempting this, regards the excitation of the cells of the respiratory centre as dependent upon a complex heterogeneous equilibrium between these cells and the blood. According to this view, the cells of the respiratory centre are excited when their protoplasm becomes more acid. Like muscle and other cells, these cells are believed to be continually producing lactic acid, which is normally being removed, chiefly by oxidative processes, but partly by passing into the blood. There would thus be a state of balance between the rate of production and the rate of removal of the acids (lactic and CO_2) in the nerve cells, and on this balance would depend the reaction, and through that, the excitability, of the respiratory centre cells. A lowered oxygen tension in the cells of the centre would lead to a diminished rate of oxidative removal of lactic acid (as with any other cells), and consequently to its accumulation in the cells, which would cause their H-ion concentration to rise. If the oxygen utilisation of the nerve cells is abruptly checked, as by the injection of a trace of cyanide into the blood, the same thing would happen at once, and there would be an instant hyperpnœa, though it may take a minute or two before there would be an increase of the lactic acid content of the blood. Addition of lactic acid or carbon dioxide to the blood, with increase of its hydrogen-ion concentration, would also indirectly affect the reaction of the cells of the centre. In the long run the addition of any strong acid to the blood is equivalent to addition of carbon dioxide, since it displaces that gas from the bicarbonate of the plasma ; carbon dioxide diffuses so readily through the walls of all animal cells that it very speedily affects the hydrogen-ion concentration of the cell interior, and it is probably this high penetrating power which explains the dominant *rôle* played by carbon dioxied in regulating the respiration.

This view certainly brings under one head all the several factors which we know to act upon respiration, namely, tension of carbon dioxide, presence of acids in the blood and considerable diminution of oxygen supply. It will, however, need considerable modification in view of the recent work on the peripheral action of chemical alterations in the blood, as outlined above. If we regard the excitable structures as consisting of a central part, the respiratory centre, and a peripheral part, the carotid body and aortic region, the former sensitive to increase of CO_2, the latter to both an increase of CO_2 and a diminution of oxygen, and substitute the metabolic changes in the peripheral structures for those in the centre, the theory would probably be adequate provisionally to explain most of the facts now known.

Since the animal has developed a mechanism by which the reaction of the blood can be rapidly adjusted by varying the excretion of carbon dioxide, whilst the excretion of other acids is relatively slow, carbon dioxide may be regarded, as in effect, the normal respiratory stimulant.

* Campbell, Douglas and Hobson. *J. Physiol.*, 1914, 48, 303.

Influence of Blood Pressure Changes. Carotid Sinus. It has long been known that the activity of the respiratory centre is modified by alterations in the general arterial pressure. Thus, the occlusion of both carotid arteries leads to a rise of arterial pressure, and to stimulation of respiration. It has been shown by Heymans and Bouckaert * that this effect is due to the reduction of pressure in the carotid sinus, since it disappears if the sinuses are denervated (Fig. 504). They also find that alterations of pressure in the carotid sinuses, when these are isolated from the general circulation and separately perfused, lead to important changes in the respiratory rate (*v.* Fig. 503). A rise of the sinus pressure leads to inhibition of respiration, and a fall to acceleration. The afferent nerve for this reflex is the branch of the glossopharyngeal which supplies the sinuses, but the vago-

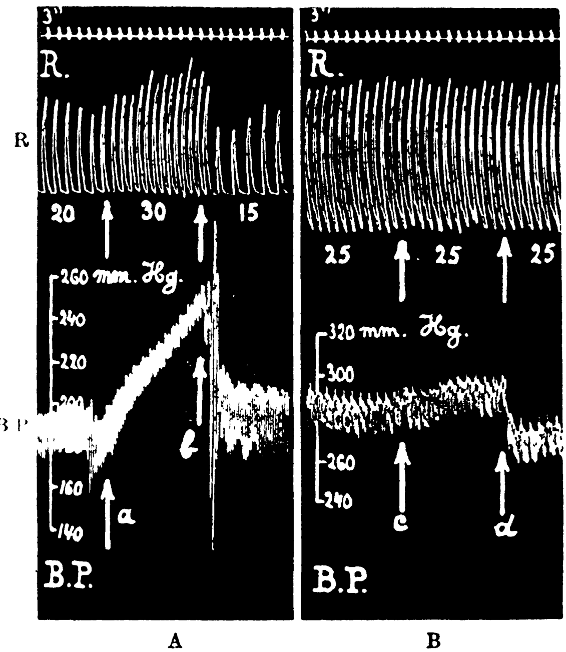


FIG. 504. Effect on respiration (R) of alterations of blood pressure of dog produced by occluding both carotid arteries at *a*, in Fig. A, and at *c* in Fig. B. The arteries were released again at *b* and *d*. In Fig. B the carotid sinuses have been denervated, which has caused a rise of respiratory rate by removal of tonic impulses from the sinuses, but change of pressure in the sinuses now produces no further effect. (HEYMANS and BOUCKAERT, *J. Physiol.*, 1930, **69**, 254.)

depressor nerves also collaborate with it, as in the regulation of the blood pressure and heart rate, and produce similar effects.

The nerves of the carotid sinuses must therefore be included with the vagi as representing those nerves which exert a constant effect on the respiratory rhythm.† After section of the carotid sinus nerves the respiration is usually depressed. This is because the accelerating impulses arising from stimulation of chemical receptors in the carotid bodies are cut off.‡ If, as in Fig. 504, these are not in action, then section of the carotid sinus nerve,

* HEYMANS and BOUCKAERT. *J. Physiol.*, 1930, **69**, 254.
† DANIELOPOLU, MARCOU and PROCA. *J. de Phys. et de Path. Gen.*, 1932, **30**, 632.
‡ STELLA. *Arch. int. Pharm.*, 1936, **53**, 289.

by removing inhibitory impulses due to pressure receptors, causes acceleration. We cannot suppose that these reflexes from the carotid sinuses are the only means by which changes in general arterial pressure may affect the respiratory centre, for unquestionably any change which leads to an alteration in the blood supply to the brain will also affect it.*

APNŒA. If artificial respiration be maintained in an animal, or if, in man, the respiratory movements be forced, so as to produce a somewhat greater ventilation than is effected by the normal respiratory movements, a standstill of respiration is brought about. This condition is called apnœa. The standstill cannot be due to over-oxygenation of the blood, because this is normally practically saturated with oxygen, and further, because a short apnœa can even be produced by excessive artificial ventilation with nitrogen.† It is due to the fact that forced ventilation of the lungs, with air or any inert gases, will reduce the carbon dioxide tension in the blood circulating round the pulmonary alveoli, and therefore round the respiratory centre.‡ A respiratory pause will thus ensue, and will last until the increasing accumulation of carbon dioxide in the blood raises its tension to the normal height at which the respiratory centre is 'set,' so to speak, to respond. If the carbon dioxide content of inspired air be increased to about 4·5 per cent., it is impossible to produce an apnœic pause, however rapidly the respiratory movements be carried out. It would seem, therefore, that ordinary apnœa is entirely due to deficiency of carbon dioxide tension in the blood ('acapnia').

It should be noted that over-ventilation and apnœa cannot be caused by artificial respiration by Schafer's method in the conscious human subject. Indeed, if the subject be brought into an apnœic state by voluntary over-breathing just before applying artificial respiration, it can easily be shown that the same procedure as will ordinarily produce a normal amount of ventilation now induces but little until the state of apnœa has passed off.§ The reason for this is that the Hering-Breuer reflex is now abnormally sensitive, so that the smallest collapse of the lungs is sufficient to check any further expiratory response and *vice versâ*, so that the movements of artificial respiration are reflexly resisted.

Stoppage of respiration is sometimes caused reflexly. A familiar example of this is the "*deglutition apnœa*" seen in man during swallowing. It lasts for about 1·5 seconds as a rule, but shows individual variations, and is shorter for swallowing of liquids than of solids. Deglutition generally occurs during the phase of expiration, which is afterwards resumed at the point at which it was arrested.‖ Similarly, when a duck plunges, there is immediate stoppage of respiration, which may last four or five minutes if the animal remains so long under water. The same stoppage may be produced by pouring water on the beak.

BREATH-HOLDING AND OTHER TESTS. After a deep expiration, followed by the deepest inspiration the breath can be held for an average time of about 68 seconds in fit men,¶ though the actual times range from 30 to 120 seconds. When the "breaking-point" is reached, it is chiefly because of the accumulation of carbon dioxide in the blood rather than because of lack of oxygen, but the differences are partly due to psychological causes. The alveolar air at the breaking-point contains about 7 per cent.

* SCHMIDT. *Amer. J. Physiol.*, 1932, **102**, 94, 119.

† HALDANE and POULTON. *J. Physiol.*, 1908, **37**, 390.

‡ HALDANE and PRIESTLEY, *J. Physiol.*, 1905, **32**, 258; DOUGLAS and HALDANE, *J. Physiol.*, 1908, **38**, 404.

§ YANDELL HENDERSON, *J. Amer. Med Assoc.*, 1914, **42**, 1133; LILJESTRAND *et al. Skand. Arch. f. Physiol.*, 1913, **29**, 149.

‖ CLARK. *J. Physiol.*, 1920, **54**, lix.

¶ SCHNEIDER. *Amer. J. Physiol.*, 1930, **94**, 464.

CO_2 and 10 per cent. O_2. Previous forced breathing will enable the breath to be held longer, as also will the filling of the lungs with pure oxygen. In the latter case the alveolar CO_2 may be 8 per cent. or more.* Conversely, the period is much shortened if the lungs be previously over-ventilated with nitrogen or with oxygen containing 5 per cent. CO_2.

The " endurance " test or " 40 mm. test " is carried out as follows : the subject's nose is clipped, he takes the deepest possible expiration, followed by the deepest possible inspiration ; the mouth is then applied to a tube connected with a mercury manometer, and he raises the mercury to a height of 40 mm. which is maintained as long as possible, the cheeks and lips being supported by the hand so that they take no part in the process. The pulse rate is counted meanwhile. A fit subject should maintain for 52 seconds, and show no great increase in pulse rate (or arterial pressure) until the point of discomfort is reached. Unfit persons maintain for a shorter time, and show a rise of arterial pressure and pulse rate, at an early stage of the test. Another test is the maximum force of expiration following a deep inspiration, as tested with a mercury manometer ; it should be 110 mm. Hg. These tests, with the requirement of a vital capacity of 3,800 c.c. are employed for the selection of recruits for the Royal Air Force.

The value of the " 40 mm." test is that it tests the tone of the abdominal muscles ; when this is poor, the intra-abdominal pressure forces out blood from the abdominal area, and increases the return to the heart, so that the pulse rate and arterial pressure rise.†

DYSPNŒA. We are not generally aware of an increase in the volume of the pulmonary ventilation until this has been about doubled. With further increase the consciousness of respiration steadily obtrudes itself, until by the time the ventilation has been increased to about four or five times the normal resting value, there is real distress, and we say that dyspnœa has set in. Dyspnœa is seen during strenuous exertion, in conditions of oxygen lack, and in certain diseased states.

The dyspnœa of exercise is associated with deficient oxygenation and increased carbon dioxide content of the blood, and these in turn may be said to be due to an improper adjustment between circulation and respiration. Usually it is the heart which is at fault, and which is the limiting factor in athletic performances of endurance ; the heart is unable to pump the blood through the lungs quickly enough, so that it becomes increasingly venous and thus excites the respiration more and more.

If vigorous exercise is continued, there comes a time when the discomfort disappears, and the subject gets what is called " second wind." It is found that at this point there is a sudden fall in the carbon dioxide content of the alveolar air ; during the dyspnœic period this may have been as high as 7·5 per cent., which often falls after second wind to 6·0 per cent. At the same time there is a fall in the volume of air breathed, a rise of temperature, and onset of sweating.‡

The phenomenon has not received a full explanation ; it is probable that it represents the attainment of a steady state, in which the circulation rate, the chemical composition of the blood, the psychical condition of the individual, the state of the respiratory centre, of heat regulation, &c. reach new equilibria. A factor of great importance is no doubt the neutralisation of the plasma bicarbonate by lactic acid set free into the blood stream ; in the early stages of exercise this is proceeding rapidly, with the result that extra carbon dioxide is being liberated from the blood. As we have seen in an earlier

* Hill and Flack. *J. Physiol.*, 1908, **37**, 77.

† Flack and Burton. *J. Physiol.*, 1922, **56**, l. ; M. R. C. *Special Rep.*, 1920, **53**.

‡ Cook and Pembrey. *J. Physiol.*, 1913, **46**, 429.

chapter, the accumulation of lactic acid in the blood leads to an increased velocity of its removal, ending in the attainment of a steady state. When this has been reached, lactic acid ceases to accumulate, and to expel carbon dioxide from the blood, which may account for the sudden fall in its output.

The dyspnœa in normal subjects associated with oxygen lack will be specially considered later.

Pathological causes of dyspnœa can only briefly be mentioned. Apart from those, such as pneumothorax, obstruction to the air-passages, &c., which are due to purely mechanical causes opposing the entry of air, the chief types are those seen in renal disease, cardiac disorders, diabetes and anæmia. In renal disease* and in diabetes, there is an excess of fixed acids in the blood, raising its hydrogen-ion concentration. In cardiac cases there is not only a reduced circulation rate, but usually some secondary alterations in the lungs themselves, for both of which reasons there is difficulty in keeping the blood properly arterialised. In anæmia, the number of red corpuscles in the blood is insufficient to carry the proper amount of oxygen to the tissues, so that the condition is virtually the same as in other states of oxygen lack. All these conditions, however, are actually more complicated than would appear from what has been said above, and often the cause of the dyspnœa remains obscure.†

OVER-VENTILATION. (Forced Breathing.) There is a large amount of carbon dioxide, free or fixed, stored in the blood and tissues, and we may compute the whole body of an adult man to contain some 15 or 20 litres of carbon dioxide. The oxygen store of the body is held almost entirely by the hæmoglobin, and does not exceed 1 litre. Now, forced (*i.e.* voluntarily quick and deep) breathing very rapidly washes out carbon dioxide from the large store, but does not at all increase the oxygen store. By continuing forced respiratory movements for a minute or two, the carbon dioxide tension, both in the alveoli and in the blood, may be brought down to a very considerable extent.

In consequence of the lowering of carbon dioxide pressure in the blood,

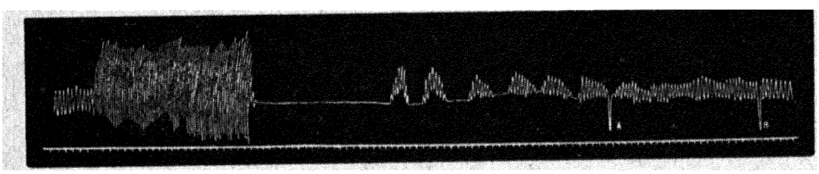


FIG. 505. Forced Breathing of Air for two minutes, followed by Apnœa for two minutes, and Periodic ('Cheyne-Stokes') Breathing for about five minutes. (DOUGLAS and HALDANE.)

At A, sample of alveolar air contained O_2, 11·44 per cent. : CO_2, 5·58 per cent. Second sample at B, O_2, 13·55 per cent. ; CO_2, 5·57 per cent.

this becomes more alkaline, and various secondary phenomena ensue. Thus, alkaline urine is excreted, and this may even contain free bicarbonate ; it also contains less ammonia than previously and may contain aceto-acetic acid.‡ The bicarbonate of the blood plasma is lowered,§ partly by reason of the ionic interchange with the corpuscles, partly by excretion of base. The blood lactate is increased, because glycolysis in the blood is accelerated. The calcium ion concentration of the blood is lowered, and in consequence symptoms of tetany may appear. The rise in pH of the blood, together

* HENDERSON *et al.* *J. Biol. Chem.*, 1927, **75**, 305.

† CAMPBELL, HUNT and POULTON, *J. Path. and Bact.*, 1923, **26**, 234 ; HENDERSON, "Blood," 1928, p. 265 (Yale Univ. Press) ; DAUTREBANDE, "Les échanges respiratoires," 1930, pp. 105–282 (Paris : Press Universitaires de France).

‡ DAVIES, HALDANE and KENNAWAY. *J. Physiol.*, 1920, **54**, 32.

§ HENDERSON and HAGGARD. *J. Biol. Chem.*, 1918, **33**, 333 ; DALE and EVANS, *J. Physiol.*, 1922, **56**, 125.

with the fall of CO_2 pressure affects the vasomotor centre, causing a fall of arterial pressure,* and also depresses the respiratory centre. As a result, there is a prolonged period of apnœa. During this period of cessation of respirations, however, the oxygen is being used up, and the tension of this gas in the blood may fall to such an extent that the respiratory centre is excited by lack of oxygen before the carbon dioxide tension in the alveoli has risen to its normal value. As a result of the excitation by oxygen lack, a few breaths are taken, the carbon dioxide tension is further lowered, and the stimulation due to the oxygen lack disappears. There is thus again a cessation of respiration (Fig. 505).

'CHEYNE-STOKES' BREATHING. These periods of cessation alternate with periods of respiration, so that we get a condition of periodic breathing, which is spoken of as Cheyne-Stokes respiration.† During the period of apnœa resulting from forced breathing, the great diminution of oxygen tension in the alveoli is shown by the fact that the subject of the experiment becomes blue, and may indeed lose consciousness. There are, at the same time, rhythmic changes in the blood pressure, which rises towards the ends of the periods of the apnœa, falling during the periods of respiration. The first respiration after excessive forced breathing is due to oxygen lack. The period of apnœa may therefore be considerably prolonged, if the onset of oxygen lack be postponed by increasing the tension of this gas in the alveoli at the commencement of the apnœic period. By forcibly breathing for a period of two minutes in an atmosphere of oxygen, men have succeeded in holding their breath for as long a period as eight minutes.‡

'Cheyne-Stokes' breathing is almost invariably observed as one of the effects of exposure to high altitudes, and is then especially marked during sleep. It is often present when the activity of the respiratory centre is depressed, as in cases of uræmia or pernicious anæmia. Under these circumstances, it may be temporarily removed by administering either oxygen or carbon dioxide (in small percentage) to the patient. The oxygen improves the condition of the centre; the carbon dioxide acts as an added stimulus and rouses its activity.

ANOXIA

The name anoxia is given to the condition of oxygen lack of the tissues. Lack of oxygen in the blood is also called *anoxæmia.* Four types of anoxia are recognised, viz. *anoxic*, *anæmic*, *stagnant* and *histotoxic.*

ANOXIC ANOXIA is anoxæmia due to a lowered partial pressure of oxygen in the blood, with consequent partial desaturation of the arterial blood with oxygen. It is produced by any condition which prevents the proper oxygenation of the blood in the lungs, *e.g.* by reduced oxygen pressure in the air breathed, by diseases in which the gaseous interchange in the lungs is impaired, and in congenital heart disease due to a patent foramen ovale.

ANÆMIC ANOXIA. In this form the oxygen tension of the blood is unchanged, but the quantity of oxygen which can be made available from the blood is diminished. It is seen in hæmorrhage or anæmia, in states in which the hæmoglobin is converted into some form which will not carry oxygen, *e.g.* into nitric oxide hæmoglobin, as in poisoning by nitrous fumes, or methæmoglobin, as in poisoning by chlorates, or in carbon monoxide poisoning, in which the hæmoglobin is converted into carboxyhæmoglobin.

* HENDERSON and HAGGARD. *J. Biol. Chem*, 1918, **33**, 333; DALE and EVANS, *J. Physiol.*, 1922, **56**, 125.

† CHEYNE. *Dublin Hosp. Rep*, 1816, **2**, 217; DOUGLAS and HALDANE. *J. Physiol.*, 1909, **38**, 401.

‡ VERNON *J. Physiol.*, 1909, **38**, Proc. xviii.

STAGNANT ANOXIA is seen in conditions in which the circulation through a part or over the whole body is slowed. Thus it is seen in surgical shock, circulatory failure, and venous obstruction.

HISTOTOXIC ANOXIA results when, the oxygen pressure and content of the blood being unaltered, or even raised, the tissues, though in full receipt of blood, fail to utilise the oxygen from it. In this category may be placed the poisoning by cyanides, which paralyse the respiratory enzymes of the tissues, and probably also the toxic effects of very high oxygen pressures.

The oxygen saturations of the arterial and venous bloods in the four types of anoxia are illustrated in Fig. 506.

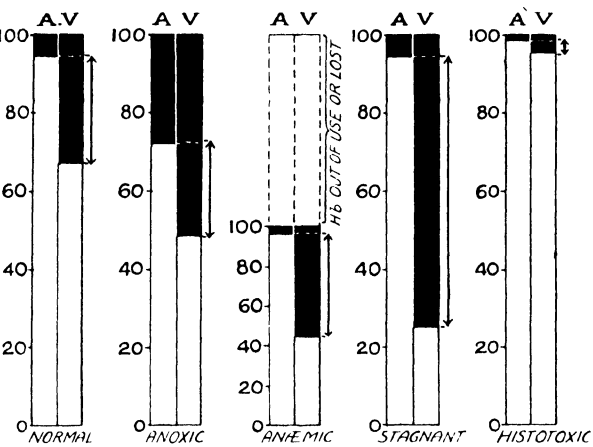


FIG. 506. Oxygen saturations of venous blood in the four types of anoxia. The black portion of each column represents the percentage of reduced hæmoglobin and the white portions the percentage of oxyhæmoglobin in the blood All the bloods had the same initial oxygen capacity when the subject was normal. In anæmic anoxia some of this, shown by dotted lines, has been lost, *e g* by hæmorrhage or by conversion to carboxyhæmoglobin. The arrows show the coefficient of oxygen utilization, *i.e* the volume of oxygen removed from one unit of blood by the tissues.

CYANOSIS

Cyanosis is the name given to a bluish colour of the skin, especially of the lips, ears, hands and feet, and of the nails. It is due to the presence of reduced hæmoglobin in the blood, and so is met with in conditions in which this occurs, whether from inadequate oxygenation of the arterial blood, or from the utilisation of an undue proportion of the oxygen of the blood during its transit through the capillary circulation, as in sluggish local or general circulation.

The degree of cyanosis depends, not on the relative amount of reduced hæmoglobin in the capillary blood, but on its *absolute amount*. To cause cyanosis there must be at least 5 g. of reduced hæmoglobin per 100 c.c. of capillary blood. Since blood normally contains about 15 g. Hb per 100 c.c., this means that below 66 per cent. oxygen saturation of the blood in the capillaries will cause cyanosis. But an anæmic person with half the normal Hb content would have to be only 83 per cent. saturated with oxygen before cyanosis was evident.

It is convenient in this connection to consider the *degree of unsaturation* of the blood. Normal arterial blood is about 5 per cent. unsaturated, which

for an oxygen capacity of 20 vols. per cent. is 1 c.c. oxygen deficit or unsaturation per 100 c.c. blood. The mixed venous blood is about 6 c.c. per 100 c.c. unsaturated, *i.e.* the tissues have taken out 5 c.c. oxygen per 100 c.c. blood. The capillary unsaturation may be taken as midway between the arterial and venous levels, *i.e.* as 3·5 c.c. per 100 c.c. Since 1 c.c. oxygen will fully saturate 0·75 g. Hb, the capillary blood will therefore contain $3{\cdot}5 \times 0{\cdot}75 = 2{\cdot}5$ g. Hb in reduced form, *i.e.* there will normally be no cyanosis.

Suppose now that we consider the blood in a subject in a state of anoxic anoxia, due, suppose, to breathing an atmosphere in which the oxygen had been reduced. The blood leaving the lungs contains, say, only 15 vols. oxygen per 100 c.c.; the tissues abstract the usual quota of 5 vols. per cent., so that the venous blood contains 10 vols. per 100 c.c. The arterial and venous unsaturations are respectively 5 and 10 c c. and the capillary unsaturation again the mean of these, viz. 7·5 vols. per cent. $= 7{\cdot}5 \times 0{\cdot}75 = 5{\cdot}6$ g. Hb. There would be cyanosis.

Again, suppose the arterial blood to be normal, *i.e.* 1 per cent. unsaturated, but owing to slow circulation the venous blood was 15 per cent. unsaturated. The capillary unsaturation would be 8 per cent $= 8 \times 0{\cdot}75 = 6$ g. Hb, and again there would be cyanosis.

An anæmic subject with an oxygen capacity of half the normal, viz. 10 vols. per cent., if exposed to the anoxic conditions would have 0·5 per cent. unsaturation in his arterial blood, and $9{\cdot}5 - 5{\cdot}0 = 4{\cdot}5$ vols. O_2 or $10 - 4{\cdot}5 = 5{\cdot}5$ vols. unsaturation per cent. in his venous blood. His capillary unsaturation would thus be the mean of 0·5 and 5·5, *i e* 3·0 c.c. $= 3 \times 0{\cdot}75 = 2{\cdot}25$ g. Hb, so there would *not* be any cyanosis.

In the reverse condition of polycythæmia, in which the number of red cells is abnormally increased, the opposite would hold good, and cyanosis would ensue on relatively slight anoxæmia.

THE EFFECTS ON RESPIRATION OF CHANGES IN THE AIR BREATHED

We have already seen that a moderate increase in the carbon dioxide content in the air breathed (*e.g.* up to 4 per cent.) causes a proportional increase in the ventilation of the lungs and that this is due to the increase of CO_2 and not to the slight diminution in oxygen content. High percentages of CO_2 cause abnormal effects leading to narcosis and death.

CHANGES IN OXYGEN TENSION. Oxygen itself exercises no effects on the respiratory movements. At the normal atmospheric pressure, the tension of oxygen in the alveoli, when air is breathed, is amply sufficient to saturate the hæmoglobin passing through the lungs. Hence no alteration in respiration will be produced by increasing the tension of oxygen in the air breathed above its normal amount.

This statement is true only for the healthy individual. If from failure of the heart and circulation, from diminished oxygen tension, or from severe loss of blood, the oxygenation of the blood is insufficient, marked amelioration of the symptoms may be produced by inhalation of pure oxygen. Especially is this noticeable where there is failure of the heart. In these cases, the heart, already affected. is unable to keep up an adequate circulation and to supply itself with sufficient oxygen. A vicious circle is thus established, in which the heart tends to get steadily worse. By administration of oxygen, an adequate supply of this gas to the heart muscle is assured; the heart beat therefore becomes more effective and the whole circulation is improved.

If a warm-blooded animal be placed in a chamber containing pure oxygen at a pressure of four atmospheres, or air at twenty atmospheres, it dies as rapidly as if it were in an atmosphere of pure nitrogen.* The explanation of the phenomenon is probably as follows: † At the high pressure enough oxygen can be taken up by the animal's plasma to supply its wants, so that the oxyhæmoglobin does not become reduced in the tissues. But then, the more acid oxyhæmoglobin retains the available base of the blood and so prevents this base from combining with CO_2 from the tissues. Hence there is a rise of CO_2 pressure within the tissues which ultimately causes death. As pointed out by Campbell, it is the rise of CO_2 pressure in the tissues which causes the convulsions that precede death under high oxygen pressures.

We have seen that the oxygen pressure in the air may be lowered to about 13 per cent. without pronounced discomfort, though there may be blueness of the lips and nails, owing to deficient oxygenation of the blood. With further reduction, hyperpnœa occurs, and, if the administration of low percentages of oxygen, *e.g.* about 10 to 12 per cent. of an atmosphere, be continued for some time, the subject of the experiment may suffer considerable discomfort. The signs of oxygen lack are severe headache, confusion of thought, vomiting or nausea and a feeling of discomfort in the precordial region. Many experiments have been made, both on animals and on man, by submitting them to a lowered atmospheric pressure in chambers specially built for the purpose. ‡ The limit to which the pressure may be reduced varies in different individuals, the variations being determined by the type of respiratory movement of the individual in question, since on the depth of respiration depends the relation between the tension of oxygen in the alveoli and that in the inspired air. The lowest limit at which life is possible corresponds to an oxygen tension in the alveoli of 27 to 30 mm. Hg.

Respiration at reduced Atmospheric Pressure. The physiology of respiration at reduced atmospheric pressures is particularly interesting in connection with exploits in mountaineering or aviation ; the chief difference between the two being that in the former great physical exertion must be coupled with the drawbacks of reduced air pressure and adverse climate, while in the latter there is the drawback that the reduction of pressure is rapid and the onset of symptoms correspondingly sudden. A good deal of investigation has been carried out for the purpose of studying the phenomena involved in the response and acclimatisation to lowered air pressure, during both aircraft § and mountain ascents. ||

The phenomena above described are exactly similar to those which are regarded as characteristic of mountain sickness. The following Table shows the diminution in the atmospheric pressure at varying heights above the level of the sea :—

At a height of 5000 metres (16,400 feet) the pressure of the air is reduced to little over half an atmosphere, and the oxygen tension is therefore only about 11 per cent. of an atmosphere. Moreover a greater *volume* of the

* PAUL BERT, *La Pression Barométrique*. 1878 (Paris); LORRAIN SMITH, *J. Physiol.*, 1899, **24**, 19.

† CAMPBELL. *J. Physiol.*, 1925, **60**, 20 ; 1929, **68**, *vii.* ; BEAN. *J. Physiol.*, 1931, **72**, 27, BEHNKE, *et al.* *Amer. J. Physiol.*, 1934, **107**, 13.

‡ BOYCOTT and HALDANE, *J. Physiol.*, 1908 **37**, 355 ; HALDANE, KELLAS and KENNAWAY, *J. Physiol.*, 1919, **53**, 181 ; SCHNEIDER, TRUESDELL and CLARKE, *Amer. J. Physiol.*, 1924, **70**, 283.

§ SCHNEIDER and CLARKE. *Amer J. Physiol.*, 1926, **76**, 354.

|| DOUGLAS, HALDANE, HENDERSON and SCHNEIDER, *Phil. Trans.*, 1913, **B203**, 185 (Pikes Peak); BARCROFT *et al.*, *Phil. Trans.*, 1922, **B211**, 351 (Andes). HINGSTON, *Geogr. J.*, 1925 **65**, 4 (Everest Exped.).

alveolar air must consist of carbon dioxide if the *tension* of this gas is to be kept nearly constant (cp. Fig. 495).

Height above sea level, (in metres)	(in feet)	Barometer mm. Hg.	Pressure in Per cent. of an atmosphere
0	0	760	100
1000	3,281	670	88
2000	6,562	593	78
3000	9,842	524	69
4000	13,123	463	61
5000	16,404	410	54
6000	19,685	357	47
7000	22,966	320	42
8000	26,246	290	38
9000	29,523	260	34

The much greater diminution in the partial pressure of oxygen than in that of CO_2 in the alveolar air is shown by the following average results obtained during fourteen aeroplane flights, with five subjects:—

ALVEOLAR AIR DURING AEROPLANE FLIGHTS. (SCHNEIDER and CLARKE, *Amer. J. Physiol.*, 1926, **76**, 354.)

Altitude, feet	0	4,000	8,000	12,000	15,000
Alveolar oxygen pressure mm. Hg .	100 5	90 5	67 2	58·1	49 8
Alveolar CO_2 pressure mm. Hg .	40 9	35 3	35·7	32 5	29 2

The carbon dioxide pressure, however, does fall somewhat, and this is due to the increased pulmonary ventilation, which shows a steady increase as the reduction of pressure progresses. If the low pressure is maintained, the breathing continues steadily to rise to a maximum ventilation some hours or days later. In the 1938 Mt. Everest expedition the alveolar partial pressure at 22,800 feet was 16·5 mm. CO_2 and 35·7 mm. O_2 after acclimatisation; at 28,000 feet (reached) there would probably be 16 mm. and 25 mm. respectively. The increased breathing is a consequence of the reduced oxygen pressure and it leads to an increased excretion of carbon dioxide, and, therefore, at first to an increase in the alkalinity of the blood, and the excretion of an alkaline urine. If the volume of air breathed be reduced by calculation to normal pressures, it is found that less air is breathed than at sea level.

This mechanism affords a means of immediate adaptation, and is also operative in the adaptation which comes on gradually during residence at a high altitude. Part of this acclimatisation appears to depend upon the excretion, by the kidneys, of bicarbonate from the blood, thus compensating for the low content of free CO_2. Under these conditions, as Barcroft has shown, the reaction of the blood returns practically to normal.

Another important means of rapid adaptation is by means of the circulation. This is noticeable even in persons sitting quietly in a chamber in which

they can be subjected to gradually lowered pressures, and no doubt holds good for aviators. It is evident that a deficient oxygenation of the blood may, so far as the tissues and heart are concerned, be compensated by increasing the rapidity of the circulation ; and this happens. The following Table shows the changes in the pulse rate caused by exposure to varying pressures in a gas chamber :—

PULSE IN CHAMBER AT REDUCED PRESSURES.

Pressure mm Hg.	Pulse p min.
720	64
650	72
424	84

This quickening of the pulse is to be observed also in the trained mountain soldier, and in individuals in whom there is no lowering of the alveolar carbon dioxide tension, so that apparently, in such cases, the whole adaptation to altered conditions is by means of the circulation. In cases where adaptation fails, it is in the circulation that the failure is most marked, so that the symptoms of severe mountain sickness closely resemble those produced by heart failure. The disturbance of the central nervous system is shown by the almost invariable occurrence of Cheyne-Stokes breathing at great heights. But this mechanism, again, is only immediate and does not explain acclimatisation to life in mountain districts. Barcroft, in the Peruvian Andes (4,400 metres) found that at rest the pulse was normal and that there was no increase in the circulation rate.

As a result of the oxygen starvation, there is inadequate supply of this gas to the heart, so that the circulation tends to fail, especially on making the slightest muscular movements. At the same time, the oxygen starvation of the brain produces failure of judgment and inability to carry out or to co-ordinate muscular movements properly. As the subject of oxygen lack is unaware of these effects, one of which takes the form of extreme stubbornness, the insidious danger of the condition is of great importance in aviation. Breathing of oxygen-enriched air is essential for pilots at altitudes over 15,000 feet, if effects of oxygen lack are to be avoided. At over 43,000 feet even this does not suffice, as the pressure is too low to give the requisite oxygen pressure ; hence the pressure must be raised by the use of either a sealed flying suit filled with oxygen under slight pressure or a sealed cockpit filled with oxygen, also under slight pressure. With such arrangements oxygen lack ceases to be a limiting factor in high flying, and heights of over 50,000 feet have already been reached.

The symptoms of oxygen lack, as a rule, do not become conspicuous until just before collapse, so that, although there is an oxygen starvation of the body, there must be some means by which the respiration is modified so as to obtain a sufficiency of this gas for the lowered requirements of the body.

But if the animal is able to withstand the immediate effects of exposure to a rarefied atmosphere, processes of adaptation come into play which finally fit him for discharging his functions normally, even at the high altitude. That the adaptation is effective, is shown by the fact that most individuals, if they remain at a height, gradually recover from the mountain sickness and may finally be able to carry out muscular movements with almost as great precision and force as they could previously on the plains. The main factor in rapid adaptation to lowered oxygen pressure is not an increase in the tissue O_2-tension, but an habituation of the heart and nervous tissues to the lowered O_2-tension.* As an immediate effect of change to a region of

* CAMPBELL. *J. Physiol.*, 1926, **62**, 211 ; 1927, **63**, 325.

low atmospheric pressure, there is an apparent increase in the blood corpuscles, due to a concentration of the blood and a diminution of its plasma, and perhaps also to a contraction of the spleen. Simultaneously, however, the reduction in the oxygen saturation of the blood is at once felt by the blood-forming organs, so that, after a stay of four or five weeks' duration at a height, both corpuscles and hæmoglobin are considerably increased in total amount. The following Table shows the average number of red corpuscles contained in one cubic millimetre of blood from the inhabitants of regions at varying altitudes :—

	Height above sea level, in metres	Red corpuscles
Christiania . .	0	4,970,000
Zurich . .	412	5,752,000
Davos . .	1560	6,551,000
Arosa . .	1800	7,000,000
Cordilleras . .	4392	8,000,000

As a result of the increase in the concentration of red cells, there would, in the later stages of acclimatisation, be an increase in the tissue O_2-tensions.* At the same time, owing to a rise in the excitability of the respiratory centre, the total ventilation is increased, so that the alveolar oxygen tension is raised 10 or 12 per cent. above that which it would otherwise be.

There is, of course, a limit to the power of adaptation, a limit which varies in different individuals. Thus, for some men, it is impossible to stay any length of time in the high settlements in the Andes, while others, after two or three weeks' discomfort, become perfectly inured to their new conditions. It seems doubtful, however, whether any of the present race of men could become adapted to permanent residence at a height over 6000 metres, though for a certain length of time, by bringing into play the reserve mechanisms already described, they may raise themselves to a height considerably above 7000 metres. The highest summits in the Himalayas (*e.g.* Mount Everest, 8865 metres), have a height approaching that attained in 1875 by Tissandier with two companions in his famous balloon ascent, namely, 8600 metres. In this ascent, although oxygen inhalation was used (somewhat ineffectively), two of the party succumbed. The expeditions to Mount Everest showed, however, that when acclimatisation was gradual, men could live at about the same altitude, *e.g.* Mallory's party reached 8200 metres (26,900 feet) without oxygen inhalation. But at such heights, the slightest exertion, *e.g.* changing from lying to sitting, caused extreme breathlessness. Oxygen often gives relief under these conditions, though its administration presents difficulties.†

Alterations in the Nitrogen Tension. The nitrogen contained in the atmosphere is a purely inert gas. It is a matter of indifference whether, under normal atmospheric pressure, we breathe an atmosphere of pure oxygen, or one containing one-fifth part of this gas diluted with four-fifths of nitrogen. The very inertness of nitrogen may be of danger to the body under certain conditions, since nitrogen is especially soluble in adipose and nervous tissues ; even when breathing air about 5 per cent. by volume of

* Campbell. *J. Physiol.*, 1928, **65**, 256.
† Lloyd. *Nature*, 1939, **143**, 961.

nitrogen is dissolved in the fat.* In all, the body normally contains about 1 litre of dissolved nitrogen, one-fifth of which can be washed out in a few minutes by breathing oxygen.* If a man or an animal be exposed, as in a caisson, to a pressure of three to six atmospheres, the small amount of nitrogen dissolved in the body is increased in direct proportion to the pressure. If the pressure be now suddenly released, the nitrogen, which cannot be used up by the tissues, is given off from the tissues in the form of insoluble bubbles. These bubbles, occurring in all the capillaries, obstruct the flow of blood, and therefore, if the evolution of gas is sufficiently large, the animal dies in convulsions. A similar evolution of gas may occur in the spinal cord, causing damage to the cord and paralysis ('caisson disease'; 'divers' palsy').† In order to prevent this sudden evolution of gas, it is necessary that the change from the high pressure to the ordinary atmospheric pressure should be carried out gradually (best of all in oxygen), so as to give the tissues time to get rid of their excess of nitrogen without the formation of bubbles.‡ Another method is to replace the atmospheric nitrogen by helium, giving the diver a helium-oxygen atmosphere, since helium is less soluble than nitrogen.§

OTHER GASES. Hydrogen and methane are, like nitrogen, indifferent gases, and may without harm be used, instead of nitrogen, to dilute the oxygen that we breathe.

Carbon monoxide is rapidly poisonous since it combines with hæmoglobin, forming CO-hæmoglobin. The blood is therefore deprived of its oxygen carrier, and the animal dies of asphyxia. The importance of CO poisoning lies in the fact that coal gas, motor car exhaust, breech gases from guns, etc., contain a high proportion of CO. Breathing air containing 0·1 per cent. of CO causes severe symptoms in 30 to 60 minutes. In such chronic poisoning with CO, there is a great lowering of the tissue oxygen-tensions.|| But, although the avidity of CO for hæmoglobin is about 300 times that of oxygen, we can convert the CO-hæmoglobin back into oxyhæmoglobin by increasing the mass influence of the oxygen and by adding CO_2. This may be done by giving the poisoned animal oxygen containing 7 per cent. CO_2 to breathe.

The blood of town-dwellers usually contains 0·1—0·2 vols. p.c. of CO.

The curve relating CO-saturation to CO-pressures is similar in form to the oxygen dissociation curve, but, as shown by Douglas, Haldane and Haldane,¶ the CO-pressures required are very small, only about $\frac{1}{245}$th as great as the oxygen pressures. Thus, we get half saturation of the blood with CO at a CO-pressure of about 0·13 mm. Hg (in presence of 40 mm. CO_2), whereas for oxygen we require a pressure of about 32 mm. (with 40 mm. CO_2). When, however, oxygen and carbon monoxide are both present, the saturating power of carbon monoxide is not 245, but 310, times as great as oxygen. This is possibly due to the fact that the velocity constant for oxygenation of hæmoglobin is about 7·5 times as great as that for reduction, together with the fact that, although the rate of combination of CO with hæmoglobin is only one-tenth as great as for oxygen, the rate of dissociation of COHb is only $\frac{1}{245}$ of that of HbO_2. Hence the stability of COHb is due, not to the speed of its formation, but to the slowness of its dissociation. (Hartridge and Roughton.**)

In cases of CO poisoning there is no dyspnœa. This is because, as we have learned,

* CAMPBELL and L. HILL. *J. Physiol.*, 1931, **71**, 309.

† HILL and MACLEOD, *J. Hygiene*, 1903, **3**, 436; HILL and GREENWOOD, *Proc. Roy. Soc.*, 1907, **79B**, 21.

‡ L. HILL and MACLEOD. *J. Physiol.*, 1903, **29**, 382.

§ END. *Amer. J. Physiol.*, 1937, **120**, 712.

|| CAMPBELL. *J. Physiol.*, 1929, **68**, 81.

¶ DOUGLAS, HALDANE and HALDANE. *J. Physiol.*, 1912, **44**, 275

** HARTRIDGE and ROUGHTON, *Proc. Roy. Soc.*, 1923, **102A**, 595; **104A**, 376, 395; 1925, **107A**, 654; 1923, **94B**, 336.

the dyspnœic response to oxygen lack is a reflex from the carotid body when the oxygen *tension* of the arterial blood is lowered. But in CO poisoning the oxygen tension of the blood is normal, though its oxygen content is reduced. The other symptoms of oxygen lack are, however, more serious than the reduction of the oxygen saturation would seem to warrant. This is because CO alters the shape of the oxygen dissociation curve, removing the S-shape, and moving it to the left, so that the blood less readily gives off oxygen to the tissues.*

Many other gases have specially poisonous properties.

Irrespirable gases are those which are so irritating that they produce spasm of the glottis. Such are ammonia, chlorine, sulphur dioxide, a high percentage (*e.g.* 20 per cent.) of CO_2, and many others.

Protection of the Lungs from Dust. Although the inhaled air contains dust particles, very few of these reach the deeper structures of the lung, and those that do so are quickly removed. Coarser dusts are normally removed from the air as this passes over the extensive nasal mucosa, which is always kept moist with sticky mucus. In mouth-breathing this line of protection is greatly weakened, but the trachea and bronchial tubes are similarly moistened and so most of the dust particles reaching them are trapped. These are then carried upwards in the coat of mucus, and, reaching the mouth, are swallowed. This mechanism is remarkably efficient and very few dry dust particles ever reach the alveoli. Dust particles trapped in the larger tubes are carried upwards at a speed of 1–3 c.m./min.† It has been claimed that the removal of dust-laden mucus is expedited by peristaltic contractions of the bronchial tubes synchronising with respiratory movements.

Extremely fine and scanty particles do, in spite of these defence measures, find their way into the alveoli. These are removed by wandering cells and deposited in the lymph glands at the root of the lungs. In town-dwellers and in coal workers these glands are always found to be black and to contain many cells that have carried ingested dust particles.

VENTILATION

A point of practical importance is the securing to each individual of sufficient fresh air. It is found that a dwelling-room becomes unpleasant and stuffy when the amount of CO_2 present has reached 0·1 per cent. This stuffiness is due to organic exhalations from the skin, lungs and alimentary canal. As these cannot be measured, it is taken as a cardinal rule in ventilation that the amount of CO_2 should never rise above 0·1 per cent.

Since in questions of ventilation we have generally to deal with trades in which the metric measure is not used, it may be convenient to give in cubic feet the data as to carbon dioxide production and the amount of air required.

An adult man gives off about 0·6 cubic foot of CO_2 every hour. Hence, in that time he raises the amount of CO_2 in 1000 cubic feet of air from 0·04 per cent. (the normal amount in the atmosphere) to 0·1 per cent. He must, therefore, be supplied with 2000 cubic feet of air per hour, in order to keep the amount of CO_2 down to 0·07 per cent. (Ordinary air contains 0·04 per cent. CO_2, therefore 2000 cubic feet would contain 0·8 cubic foot CO_2, which, with the 0·6 cubic foot given off by the man, would be 1·4 cubic feet, which is 0·07 per cent.)

In order that the air may be easily renewed without giving rise to excessive draughts, a certain amount of cubic space must be allotted per person. Each adult in a room should have 1000 cubic feet of space, and be supplied every hour with 2000 to 3000 cubic feet of air.

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* J. B. S. Haldane. *J. Physiol.*, 1912, **45**, 22 P.

† Florey, Carleton and Wells. *B. J. Exp. Pathol.*, 1932, **13**, 269; Barclay, Franklin and Macbeth. *Amer. J. Rœnt. and Radium Therap.*, 1938, **39**, 673.

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BOOK VI

NUTRITION

CHAPTER XLI

THE EXCHANGES OF MATTER AND ENERGY IN THE BODY

ALL the energy which leaves the body as heat or work is derived from processes of oxidation, the carbon, hydrogen, nitrogen and sulphur of the foodstuffs being oxidised and eliminated in the form of carbon dioxide, water, urea, sulphates, etc. In a starving animal this discharge of energy must be associated with a loss of body substance. The necessity for taking food is determined by the need of replacing this loss. Most food-stuffs cannot be utilised directly as a source of energy, but must be altered and built up into the structure of the living protoplasm. The total amount of living material in the body, though fairly constant in the adult, may yet undergo alterations under some conditions, and especially in the growing animal. We have in this chapter to inquire into :

(1) The relation between the total amount of material taken up by the body and the total amount given out—the *material balance sheet ;*

(2) The relation between the potential energy available to the body and the amount of energy set free as heat or work—the *energy balance sheet :*

(3) The nature and amounts of the foodstuffs necessary for maintaining the weight of the body constant or providing for its growth and health ; and

(4) The significance of the various classes of foodstuffs as sources of energy and in the replacing of tissue waste.

1. THE MATERIAL BALANCE SHEET

METHODS EMPLOYED

INCOME. The determination of the material exchanges of the body involves comparison of its income and output. The income consists of the foodstuffs and oxygen. The foodstuffs may be divided into two classes, namely (1) the organic foodstuffs, which on oxidation may serve as source of energy, and (2) the inorganic foodstuffs, such as salts and water.

The latter class neither add to nor subtract from the total energy of the organism, but their presence is a necessary condition of all vital processes; and as they are contained in the various excreta, a corresponding amount must be present in the food in order to make good this loss.

In spite of the complexity of the foods taken by man, their essential constituents can usually be assigned to the three classes, proteins, fats and carbohydrates ; and any analysis of the food must give the relative amounts present in digestible form of these three proximate principles.

In the proximate analysis of the foodstuffs the amount of water is determined by drying the foodstuffs at 105° C., and the amount of inorganic constituents by ashing the dried remainder. The nitrogen (determined by Kjeldahl's method), multiplied by 6·25, gives approximately the total protein in the food. The total fat is determined by extracting the dried food in a Soxhlet apparatus with ether. Carbohydrates may be determined directly by boiling the food with dilute acids in order to convert all carbohydrate into hexoses, which are then estimated as glucose. The carbon and hydrogen content of the food can easily be calculated.

For approximate calculations, especially of dietaries of whole communities, the labour of analyses is lightened by the fact that nearly all the ordinary foodstuffs have been analysed and their average composition published.* In order to attain greater accuracy, some observers have made a complete food in the form of biscuits or of preserve, which is prepared in large quantities at the beginning of the experiment and used as the sole diet throughout the experiment. The monotony of such a diet tends to interfere with the success of the experiment, since the subject loses his appetite.

OUTPUT. The material output of the body is turned out by the kidneys, the alimentary canal, the lungs and the skin. These excreta must therefore be collected and analysed. In most cases the losses by the shedding of the cuticle, hairs and nails, and so on, are so small that they may be disregarded.

The nitrogen of the foodstuffs and that derived from the disintegration of the tissues of the body is excreted almost exclusively in the urine, a small amount being thrown out by the alimentary canal. The total nitrogen must therefore be determined both in the fæces and in the urine. The nitrogen in the fæces is derived from two sources. Part represents those nitrogenous constituents of the diet which have resisted digestion, and a certain amount is derived from the intestine itself. Even during complete starvation fæcal matter is formed in the intestine, and it has been calculated that in a normal individual about 1 gramme of nitrogen a day is excreted in the digestive secretions and by the mucous membrane of the gut and contributes to the formation of the fæces. It is usual, therefore, in experiments on man, to regard 1 gramme of the nitrogen of the fæces as belonging to the *output* of the body and representing the result of nitrogenous metabolism, while the balance belonging to undigested foodstuffs is subtracted from the total nitrogen of the latter in reckoning the real *income* of the body.

A small amount of nitrogen is also lost by sweat, but this can be disregarded unless the sweating is profuse. That the loss by both lungs and skin under ordinary circumstances can be disregarded is shown by the fact that it is possible to account directly for the whole nitrogen of the body by a comparison of the composition of the food with that of the urine and fæces. If, for instance, an animal is kept on a sufficient diet which contains a regular amount of nitrogen, after a few days a condition known as *nitrogenous equilibrium* is set up, *i.e.* the total nitrogen of fæces and urine is exactly equal to the total nitrogen of the food. The same applies to the sulphur, as shown in the following Table (quoted by Tigerstedt):

Days of experiment	Nitrogen of food	Nitrogen excreted	Per cent difference	Sulphur ingested	Sulphur excreted.
1-7	154·81	153 02	− 0·51	—	—
8-17	213·72	213·26	− 0·21	12·77	12·79

In order to express the nitrogenous metabolism in terms of protein, we multiply the total nitrogen of the excreta by 6·25. This will give the total protein which has been broken down during the period of the experiment.

RESPIRATORY EXCHANGE. In the determination of the total processes of oxidation of the body, information is given by a determination of the oxygen intake and of the output of carbon dioxide and water. The estimation of these substances involves the use of some form of respiration apparatus.

* R. H. A. Plimmer, "Analysis and Energy Values of Foods," H.M. Stationery Office, 1921. Atwater and Bryant, *Bull.* 28, U.S. Dept. Agricult., 1906. McCance and Widdowson, "Chemical Composition of Foods" (M.R.C., 1940).

The methods which have been used for this purpose are very numerous, but are of two main types, the closed or re-breathing type and the open or expired-air-analysis type. In the closed type, the subject breathes an atmosphere which circulates in a closed apparatus; the carbon dioxide produced is absorbed and weighed, and oxygen is added in measured amount to keep the volume of the enclosed atmosphere constant: the nitrogen is breathed over and over again. In the open type fresh air is inspired, and the expired air is subjected to measurement and analysis in one of several ways. The Benedict apparatus is on the closed principle; Haldane's and Douglas' methods are on the open principle.

I. The Method of Benedict. The animal that is to be the subject of investigation is placed in a closed chamber containing a given volume of air. The carbon dioxide produced by the animal is absorbed by means of caustic alkali, and the oxygen consumed by the animal is made good by allowing oxygen to flow into the chamber from a gasometer. The inflow of oxygen is regulated so as to keep the pressure of air in the chamber constant. At the end of the experiment, the alkali is weighed, and the amount of carbon dioxide absorbed thus determined; or the CO_2 can be expelled by acid and its volume measured direct. The air in the chamber is also analysed so as to be certain that it contains an excess neither of carbon dioxide nor of oxygen. The amount of oxygen absorbed by the animal is known already, the oxygen which has been allowed to flow in having been measured.

A modification of this method * is especially applicable to clinical purposes. In this method, the subject of the experiment breathes through a nose-piece into a wide metal tube, the mouth being kept closed. The metal tube forms part of a closed system through which a current of air is maintained by means of a blower. In the course of the current of air are interposed vessels for the absorption of carbon dioxide and of water, and the volume of gas in the system is maintained constant by admitting oxygen to it in proportion as the oxygen of the system is used up in respiration. In Fig. 507 is given a diagrammatic scheme of the air circuit. The tension equaliser is attached to the ventilating pipe near the point of entrance of the air from the lungs. It consists of a pan with a rubber diaphragm (which may be conveniently made from a lady's bathing-cap). As the air is drawn into the lungs the rubber diaphragm sinks, to rise again with expiration. The respiratory movements can thus proceed without appreciably altering the pressure within the closed system of tubes. By the admission of oxygen the supply of oxygen is adjusted so as to keep the bag from becoming either too much distended or too much flattened. As the air leaves the lungs and is swept into the constantly moving current of air, it is carried along by the pump and flows through two bottles containing strong sulphuric acid and pumice for the removal of water vapour. It then passes through a brass cylinder, filled with soda lime for the absorption of carbon dioxide. From here it passes again through sulphuric acid for the absorption of water given off by the soda lime. Since the air so deprived of moisture would be uncomfortable to breathe, it is then carried through another wash bottle containing water with a trace of sodium carbonate for the neutralisation of any acid fumes which may be given off by the sulphuric acid. It then passes back to the tube from which the subject is breathing. In this way it is possible to determine very accurately the amount of oxygen used up, and the amount of carbon dioxide given off, in the course of an experiment lasting one to three hours, or longer. The oxygen consumption is measured by weighing the cylinder of this gas, chosen small for this purpose, before and after the experiment, or else by running it in through a meter.

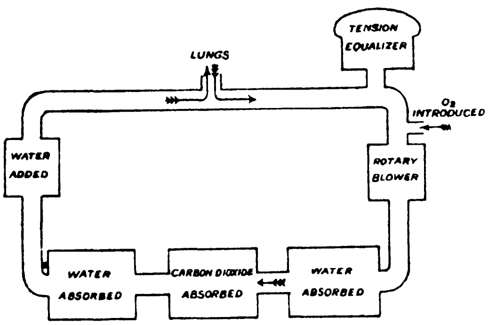

FIG. 507. Air Circuit in Benedict's Respiration Apparatus.

The Atwater-Benedict Respiration Calorimeter, on a similar principle, is described on p. 508.

* BENEDICT. *Boston Med. & Surg. J.*, 1918, **178**, 667; 1920, **183**, 449.

II. The Recording Spirometer. The Knipping apparatus (Fig. 508) is a more elaborate form which enables CO_2 production also to be measured. The air is circulated by a blower P; the subject breathes through the mouthpiece M and the CO_2 is absorbed in KOH solution. The descent of the spirometer bell S gives a measure of

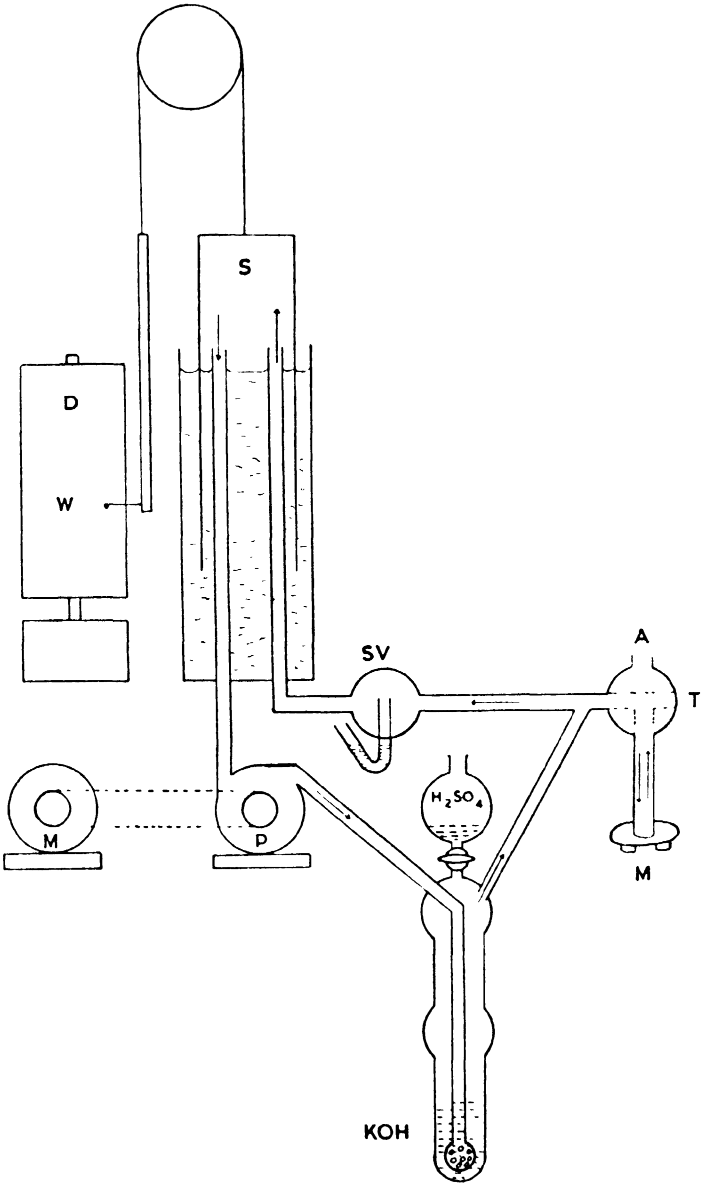

FIG. 508. Knipping Recording Spirometer. (C. F. Palmer & Co.)

the oxygen usage. As oxygen is used, the respiratory record is written on a sloping base line; more oxygen is added through a gas meter from time to time to restore the respiratory base line. The rate of oxygen usage at any time is given by the slope of the respiratory tracing. After the oxygen usage has been determined the tap T is closed and the CO_2 liberated from the KOH by adding acid from the tap-funnel. After cooling, the volume increase represents the CO_2 liberated.

III. The Method of Haldane.* This method is convenient when dealing with the gaseous exchanges of small animals, such as mice, rats, or frogs. The animal is placed in the chamber C (Fig. 509). On the inlet side of the bottle is a series of tubes or bottles, some of which contain sulphuric acid and pumice stone, while the others contain soda lime. On the outlet side of the vessel is a corresponding series of vessels

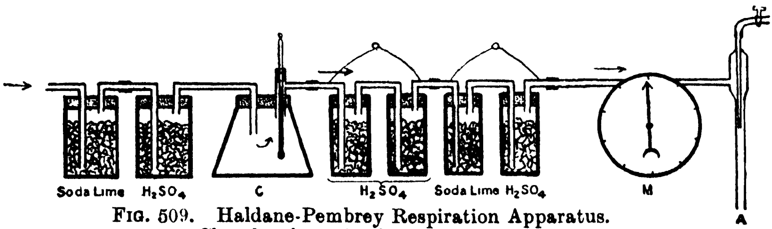

FIG. 509. Haldane-Pembrey Respiration Apparatus. C. Chamber for animal. M. Gas meter.

for the absorption of water and of carbon dioxide. During an experiment air is sucked through the whole apparatus by means of a water pump. The animal is thus supplied with pure air freed from water vapour and from carbon dioxide. All water or carbon dioxide produced by the animal is absorbed by the vessels interposed on the course of the outgoing air. These vessels are weighed at the beginning and at the end of the experiment, and the difference in weights will therefore give the amounts of carbon dioxide and water which have been discharged by the animal.

The intake of oxygen by the animal is determined indirectly. Since it gives off only carbon dioxide and water, and absorbs only oxygen during its stay in the chamber, the loss of weight of the animal during its stay in the chamber, subtracted from the total weight of carbon dioxide plus water it gives off, will represent the weight of oxygen absorbed.

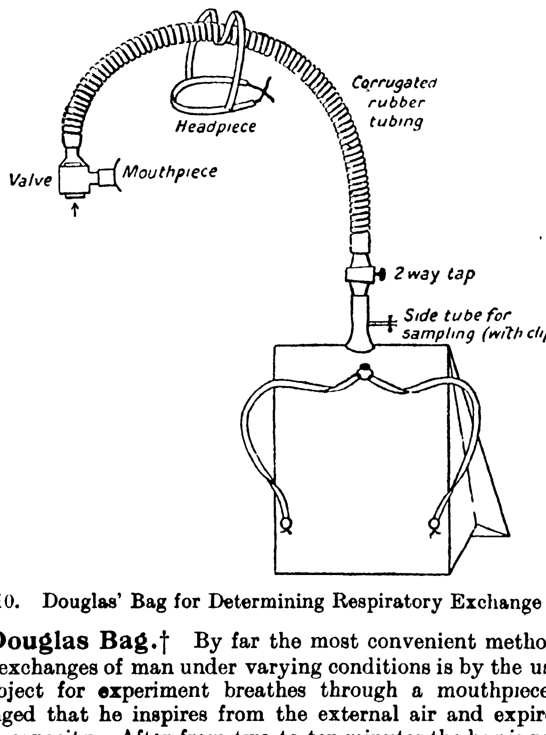

FIG. 510. Douglas' Bag for Determining Respiratory Exchange in Man.

IV. The Douglas Bag.† By far the most convenient method for estimating the respiratory exchanges of man under varying conditions is by the use of the Douglas Bag. The subject for experiment breathes through a mouthpiece provided with valves so arranged that he inspires from the external air and expires into a bag of about 100 litres capacity. After from two to ten minutes the bag is removed, the time being accurately noted. The amount of air expired during this time is measured by

* HALDANE. *J. Physiol.*, 1892, **13**, 419.
† DOUGLAS. *J. Physiol.*, 1911, **42**, *Proc.* xvii.

emptying the bag through a gas meter. A sample of its contents is analysed and the oxygen and CO_2 in it determined. Since the composition of the external air is known, the analysis and measurement of the expired air gives the respiratory metabolism during the time of the observation. The bag is carried on the back of the individual, so that it does not interfere with his movements. This method has been used for determining the metabolism of soldiers in training, of industrial workers, athletes, &c.

Calculation of Results. In the closed circuit methods, the weight or volume of oxygen used and carbon dioxide produced are given directly, and any necessary corrections, *e.g.* of volumes to dry volumes at N.T.P., are obvious. With the open methods, some explanation is necessary. The subject inspires ordinary air which contains 20·96 volumes per cent. of oxygen, 79 per cent. N_2, and 0·04 per cent. CO_2, and it is the volume and composition of his expired air during a given interval which is actually determined. As regards CO_2, let us suppose that the expired air contains 4·10 per cent., *i.e.* an increase of 4·06 per cent.; then, if his volume of expired air is 8·5 l. per min., the volume of CO_2 expired is $85 \times 4{\cdot}06 = 345$ c.c. per minute. The oxygen is less direct because, as explained in Chapter XL., the expired air has generally a different, mostly a smaller, volume than the inspired, the *percentage* of nitrogen being raised owing to utilisation of oxygen which is not converted to CO_2. The *total amount* of nitrogen breathed out is, however, equal to that inspired. Hence, if the expired air contains, say, 79·5 per cent. N_2 and 16 4 per cent. oxygen, then each 100 c.c. expired air corresponds to $\frac{100 \times 79\ 5}{79} = 100\ 6$ c.c. inspired air and this contained $\frac{20\ 96 \times 100\ 6}{100}$ $= 21{\cdot}09$ c.c. oxygen, so that the oxygen utilised per minute was $(21{\cdot}09 - 16\ 4) \times 85$ $= 398$ c.c.

The Respiratory Quotient is obtained, when required, from the respiratory exchange. It is the ratio of the volume of carbon dioxide produced during a given time to the volume of oxygen utilised during the same time. (Both gases should, of course, be measured at the same temperature and pressure.) In the above example it is $\frac{345}{398} = 0{\cdot}87$.

If the R.Q. is exactly 1·0, the percentage of nitrogen is unaltered. When the R.Q. is greater than unity, the percentage of nitrogen in the expired air is less than that in the inspired air, but the same type of calculation as that given above can be applied.

By these methods we may arrive at a correct idea of the total income and output of an individual for periods up to many days. The following details by Atwater and Benedict may serve as an example of the results obtained in such experiments:

SUMMARY OF INCOME AND OUTPUT OF NITROGEN, CARBON AND HYDROGEN IN MAN (ATWATER AND BENEDICT *)

State	Total No of Days	Average Daily Income. (Grams.)	Average Daily Output. (Grams)					Gain or Loss (Grams).
		Food.	Fæces.	Urine	Water	Respn.	Total.	
Rest	42	NITROGEN. 18 5	1·1	17 8	—	—	18·9	−0·4
		CARBON. 253·3	9·9	12 4	—	217 9	240·2	+13·1
		HYDROGEN. 39·2	1·4	3 5	41·8	—	46·7	−7·5
		(Protein lost by body = 2·4 grams) (Calc. from N. loss)		(Fat gained by body = 19 2 grams) (Calc. from C. gain)				
Fast of 1 or 2 days	5	NITROGEN. 0 0 .	0·0	13 1	—	—	13·1	−13·1
		CARBON. 0·0 .	0 0	9·9	—	184·4	194·3	−194·3
		HYDROGEN. 0·0 .	0·0	2·7	98·3	—	101·0	−101·0
		(Protein lost = 82·0 grams) (Fat lost = 198·3 grams)						

* ATWATER and BENEDICT, 1903. *U.S. Dept. Agric. Bull.*, 1903, *No.* 136, p. 120.

In the resting experiments, in which the subject was on an ordinary diet, it is seen that the intake and output almost balance ; there was, however, a slight loss of 0·4 gramme of nitrogen, which means that about 2·4 grammes of protein from the body had been lost. Some fat had, however, been laid on, as shown by the gain of 13·1 grammes of carbon. In the fasting experiment the body, of course, lost considerably.

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2. THE ENERGY BALANCE SHEET OF THE BODY

INCOME. The energy income of the body is measured by the potential energy of the foodstuffs, *i.e.* the amount of energy which can be evolved,

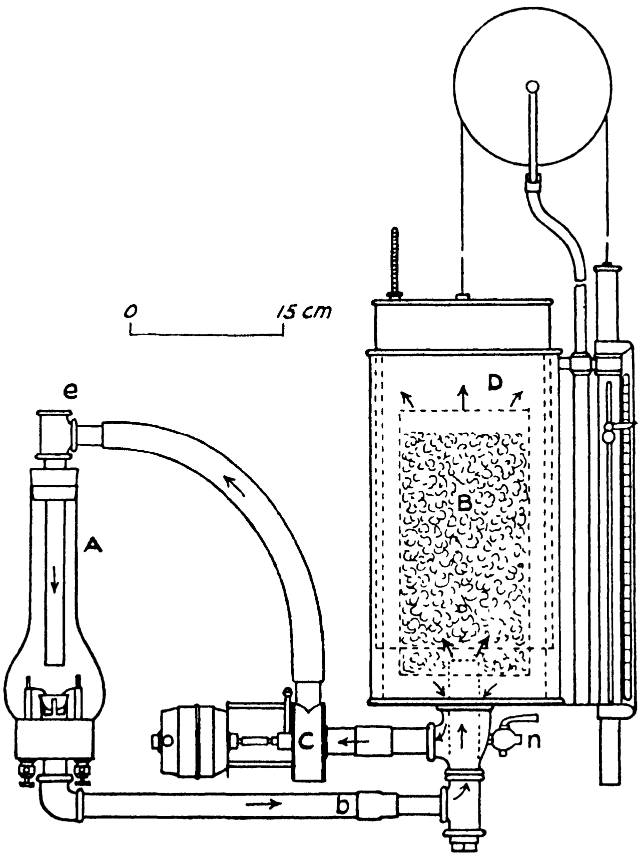

FIG. 511. Simplified oxycalorimeter for determining energy values of foods, foodstuffs and excreta. (BENEDICT and FOX, *Journ. Biol. Chem.*, 1925, **66**, 783.)

either as heat, work or in any other form, by the oxidation of the foodstuffs to the end products which occur in the body. We generally express the total potential energy of a foodstuff in *Calories*. The heat value of any given food is the number of Calories † which it evolves on complete com-

† A calorie is the amount of heat necessary to raise a gramme of water from 15° C. to 16° C. A large Calorie (printed with a capital C) is equal to 1000 small calories.

bustion with oxygen, and is determined by burning a weighed quantity of the dried foodstuff in oxygen in the bomb calorimeter.

Instead of using the bomb calorimeter, the heat of combustion can be determined *indirectly* by finding how much oxygen is needed to burn the foodstuff. As seen from the table below, for common foodstuffs the Calories yielded per litre of oxygen used only vary from 4·6 for protein to 5·06 for starch. The determination is carried out in a modified closed-circuit respiration apparatus, called an oxy-calorimeter, in which the substance is burned in a circulating current of oxygen. A suitable apparatus is shown in Fig. 511.

The substance to be tested is dried and placed in a small nickel crucible, inside the lamp-glass A. The apparatus is filled with oxygen, circulated by the rotatory blower C. The spirometer bell D gives the variation in volume, and is filled with soda lime to absorb CO_2. Combustion is started by electrical heating of the substance, and, after completion and cooling, the oxygen used is read off. Except when very accurate results are needed, an exact analysis of the substance is not necessary; the average calorific values of oxygen may then be taken to be: for nitrogen-rich substances, 4·68 Calories per litre of oxygen; for fats, 4·7 Calories; for carbohydrate-rich foods, 5·0 Calories; for a mixed diet of carbohydrates and fats, 4·825 Calories; and for fæces, 5·0 Calories.

The following heat values have been obtained for different foodstuffs:

Gaseous and Energy Relationship in the Combustion of Some Commonly Metabolised Compounds. (Benedict and Fox, *Journ. Biol. Chem.*, 1925, **66**, 783.)

Substance	Oxygen required to oxidise 1 gm	Produced in the oxidation of 1 gm		Calories per litre of oxygen
		Carbon dioxide	Heat	
	c.c.	c.c.	cals.	
Starch	829 3	829 3	4 20	5 06
Cane sugar	785 5	785 5	3 96	5 04
Glucose	746 2	746 2	3 74	5 01
Lactic acid	745 9	746 0	3 62	4 85
Animal fat	2013 2	1431 1	9 50	4 72
Human fat	1990 8	1420 4	9 54	4 79
Protein	956 9	773 8	4·40	4 60
Acetone	1542 9	1157 2	7 43	4 82
β-Oxybutyric acid	968 2	860·7	4 69	4·85
Ethyl alcohol	1459 5	972 9	7 08	4 85

In the case of some foodstuffs it is necessary to draw a distinction between the heat value on combustion, and the physiological heat value. Since carbohydrates and fats undergo complete oxidation in the body to carbon dioxide and water the values of these foodstuffs to the organism are identical with their absolute heat values. When proteins are oxidised in the bomb calorimeter, however, the nitrogen is set free in a gaseous form. In the animal body no nitrogen is eliminated in the gaseous form, but it is excreted as urea and allied substances still endowed with potential energy, which can be set free when their oxidation is completed in a calorimeter. In order to determine the physiological heat value of protein, we must subtract from its absolute heat value the heat value of the excretory products as they

leave the body. The physiological heat value of proteins was determined by Rubner in this way: A dog was fed with the same protein which had served for the determination of the absolute heat value, and its urine was collected, dried, and its heat value determined by combustion in the calorimeter. It was found that, for each gramme of protein which had undergone disintegration in the body, urine was passed of a heat value of 1·0945 Calories. The heat value of the fæces formed under the same diet was 0·1854 Calorie for each gramme of protein. Rubner further reckoned that a certain amount of heat would be required for the solution of the proteins and of the urea, and reckoned this at 0·05 Calorie. The reduced or physiological heat value of protein is therefore equal to $5{\cdot}345 - (1{\cdot}0945 + 0{\cdot}1854 + 0{\cdot}05) = 4{\cdot}015$ Calories.

It is usually unnecessary to reckon out accurately the relative values of the different kinds of protein, carbohydrate, &c. contained in each diet, and Rubner calculated the *average* physiological heat values of the three classes of foodstuffs, and found them as follows:

1 grm. protein	= 4 1	Calories
1 grm. fat	= 9·3	,,
1 grm. carbohydrate	= 4·1	,,

These figures are accurate only for a diet containing the normal proportion of vegetable to animal foods—60 to 40. The heat value of vegetable protein is as a rule less than that of animal protein.

Careful experiments have shown that the sum of the energies put out by the body is equal to the sum of the energy obtained by the oxidation of the tissues and of the foodstuffs in the body during the same time. Here are the figures from one such experiment by Atwater on man:

Date	(a) Heat of combustion of food eaten	(b) Heat of combustion of fæces.	(c) Heat of combustion of urine.	(d) Estimated heat of combustion of protein gained (+) or lost (−)	(e) Estimated heat of combustion of fat gained (+) or lost (−)	(f) Estimated energy of material oxidised in the body, a − (b + c + d + e)	(g) Heat determined.	(h) Heat determined greater (+) or less (−) than estimated. g − f	(i) Heat determined greater (+) or less (−) than estimated. h − f
	Cals	Cals	Cals	Cals	Cals	Cals	Cals	Cals.	%
Dec. 9–10	2519	110	142	−85	+3	2349	2414	+65	+2·8
10–11	2519	110	133	−25	−44	2345	2386	+41	+1·7
11–12	2519	110	132	−21	−93	2391	2413	+22	+0·9
12–13	2519	110	133	−14	−55	2345	2375	+30	+1·3
Total 4 days	10,076	440	540	−145	−189	9430	9588	+158	
Average one day	2519	110	135	−36	−47	2357	2397	+40	+1·7

OUTPUT. The energy expenditure of the body is measured in Calories and can be determined either directly by placing the subject in a suitable calorimeter, or indirectly from the oxygen consumption.

THE RESPIRATION CALORIMETER. The Atwater-Benedict * respira-

* ATWATER and BENEDICT, *A Respiration Calorimeter. Publ No. 42, Carnegie Inst., Washington,* 1905.

tion calorimeter consists of a room or chamber with double non-conducting walls. All round the inner wall of the room are fitted coils of pipes through which a stream of water flows. The pipes are fitted with discs in order rapidly to take up heat produced in the room. The current of water is accurately adjusted so as to maintain the temperature of the inner wall constant. As the inner wall and outer wall are kept at the same temperature, no heat is lost to the exterior, the whole of the heat produced by the animal or individual in the chamber being communicated to the water passing through the chamber. The temperatures of the entering and leaving water are taken by thermometers reading to a thousandth of a degree Centigrade. Knowing the amount of water that has passed through in a given time and the difference in temperature during the same time, it is easy to calculate the amount of heat given off by the animal under investigation. It is generally convenient to maintain a constant difference of temperature between the entering and leaving water, by appropriate adjustment of the amount of water passing through the apparatus. The equality of temperature between

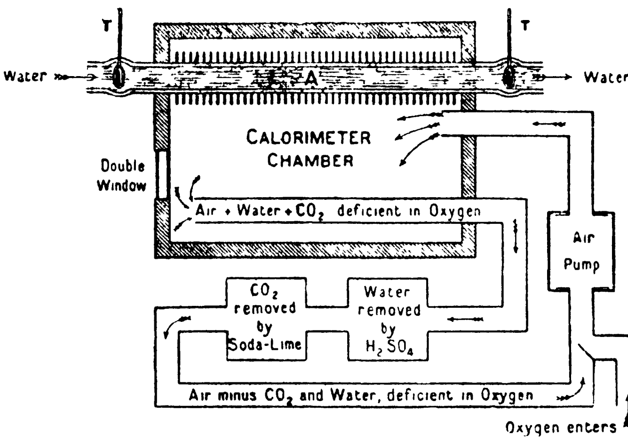

FIG. 512 Diagram to show the Principle of the Atwater-Benedict Calorimeter. (After HALLIBURTON.)

the inner and outer casing is recorded by electric thermo-couples, any difference of temperature being at once compensated by electrically warming the cooler part. The chamber contains a bicycle or other arrangement for the performance of mechanical work. It is adequately ventilated by a current of air passing through an apparatus similar to that of Benedict, described on p. 818. Any work done is measured and can be converted into forms of heat, since 1 kg. m. = ·0024 Cal. It is thus possible to estimate simultaneously the total heat production of an individual as well as the respiratory exchange, including both carbon dioxide output and oxygen intake. The general principle of the calorimeter is shown in the diagram (Fig. 512). The calorimeter is also supplied with bed, table, chair, &c., and food can be introduced through a double window so that an experiment may be continued over several days on one and the same individual.

INDIRECT CALORIMETRY. From the equation

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$$
$$\text{(180 grams) (134 litres) (134 litres)}$$

and from the observation that 1 gramme of glucose on combustion yields 3·7 Calories of heat, we can deduce that when carbohydrate is being burnt in the body (*i.e.* when the R.Q. = 1·0), the utilisation of each litre of oxygen

generates about 5·05 Calories of heat. Similar calculations can be made for the combustion of proteins (4·69) or fats (4·74), so that in all cases the calorific value of oxygen is in the neighbourhood of 5 Calories per litre, the exact value varying according to the respiratory quotient, as shown in the following Table (Cathcart and Cuthbertson) (*v.* also p. 839):

R.Q.	Cal. per litre oxygen.
1·00	5·047
0·90	4·936
0·80	4·827
0·718	4·735

These figures have been obtained by actual comparison between respiratory exchange and calorimeter experiments on man. It is therefore now quite easy, when the oxygen usage and R.Q. are known, to calculate the total energy liberation of the body.

BASAL METABOLISM

During fasting the energy output of the body is determined (*a*) by the amount necessary to keep the body alive—*i.e.* to maintain its warmth and to furnish the energy for respiratory movements, contractions of the heart, &c.; (*b*) the energy necessary to carry out any external work that is performed.

The energy requirements under (*a*) represent the 'Basal Metabolism' of the body. It may be defined as the energy expenditure of the body in the resting state, a few hours after the absorption of the last meal and under comfortable conditions of temperature, &c. The basal metabolism, of course, varies with the size of the individual; but if a number of different animals are compared, their metabolism per kilo. is found to be greater the smaller the animal.

This is due to the fact that the chief expenditure of energy is devoted to maintaining the temperature of the body, and the smaller the animal the larger is its surface, and therewith its heat loss, relatively to its weight. The basal metabolism, therefore, is more nearly a function of the surface of the body. The surface area, S, of an animal can be found approximately by the Meeh formula *: $S = k\sqrt[3]{W^2}$, where W is the weight and k a constant for the species, *e.g.* for cat, 0·099; rabbit, 0·125; rat, 0·091; dog, 0·107; man, 0·123. (Probably 0·11 is more nearly correct for man.) A comparison of the metabolism per square metre body surface in different animals shows that it is of the same order in all cases, as is seen in the following Table. We

	Body weight.	Calories per square metre body surface per day.
Animal 1	30·40	977
„ 2	23·70	1069
„ 3	19·20	1135
„ 4	17·70	1040
„ 5	10·90	1109
„ 6	6·45	1054
„ 7	3·10	1091

may say that a warm-blooded animal requires a daily expenditure of about 700 to 1000 Calories per square metre body surface, in order to maintain its temperature and carry out such motor processes as are essential to life.

* MEEH. *Zeit. f. Biol.*, 1879, **15**, 425. For dogs, Cowgill and Drabkin's formula (modified) is better: A (in m²) = 0 2864 $W^{0\ 367}$ L, where W = weight in kg. and L = body length in m. from nose to anus (*Am. J. Physiol.*, 1927, **81**, 36).

The same thing applies to man. If we desire to predict the normal basal metabolism of any given individual we must find his surface. This depends on height as well as on weight. If we know the height and weight, the surface can be calculated by the following formula (Du Bois *) :

$$S = \cdot 007184 \times W^{0\cdot 425} \times H^{0\cdot 725}$$

where S is the surface in square metres, W the weight in kilogrammes, H the

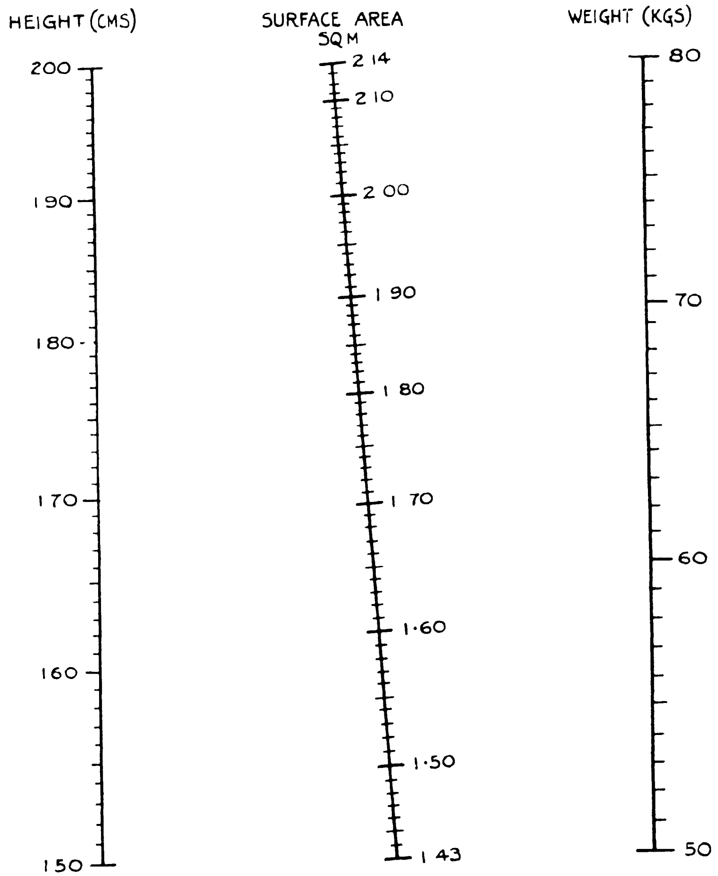

FIG. 513. Alignment chart for Calculation of Body Surface. A thread or ruler, placed so as to lie on the points for weight and height, cuts the line for surface at the correct value. (W. A. M. SMART.)

height in centimetres ; or, more conveniently, by the use of a nomogram such as is shown in Fig. 513.

In young adult white men the basal metabolism is about 40 *Calories per square metre per hour*. It is about 2·5 Calories less for women. From age forty onwards there is a reduction, the formula † being 39·138 − 0·0678 × age, Cal./sq. m./hr., *e.g.*, at fifty it would be about 35·75 Cal./sq. m./hr.

In the new-born infant, however, it is low (25 Calories per square metre per hour), and is even lower in premature infants. It rises rapidly in the first year of life, and at five years is about 52 Calories per square metre per

* DU BOIS and DU BOIS. *Arch. Int. Med.*, 1916, **17**, 863, 887.

† LEWIS. *Am. J. Physiol.*, 1938, **121**, 502.

hour. There are racial variations, due partly to varying diet and in part to different extents of muscular relaxation.

It is usual to determine basal metabolism directly by estimating the oxygen usage of the resting, fasting individual, and calculating from the calorific value of oxygen at the observed respiratory quotient. The observed value can then be compared with the value predicted from the body surface. The results are usually given in percentages of the normal predicted basal metabolism. In certain diseases the normal is departed from, a divergence of 10 per cent. being considered significant. Thus in fevers there is an increase during the febrile state, which may amount to 50 per cent. In diseases associated with over-action of the thyroid there is also an increase to 20 to 50 per cent. above normal, while in the converse condition of subthyroidism there is a reduction of 10 to 30 per cent.

Injection of adrenaline, or mental excitement increases the basal metabolism.

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Terroine et Zunz. Le Métabolisme de Base. (*Press. Univ. de France*)

3. INFLUENCE OF STATE OF NUTRITION ON METABOLISM

The basal metabolism also depends on the state of nutrition of the animal. Thus in fattening an animal, the basal metabolism increases steadily as the animal gets fatter. It requires much more food to keep a fat animal fat, than to maintain it in its original condition before fattening. The figure 40 Calories per square metre of body surface applies to a well-nourished individual. If the food normally supplied to the individual be diminished, he can go on working as before, obtaining his energy at the expense of the fat and muscular tissues of his own body. But when, under this régime, the weight has fallen about 12 per cent. it is found that the basal metabolism is also largely reduced. During the War of 1914–18, Zuntz and Loewi * found their own basal metabolisms lowered by 15 and 12 per cent. In a squad of men investigated by Benedict,† the food normally taken ranged from 3200 to 3600 Calories per day. After these men had by restriction of diet to 1400 Calories suffered a loss of 12 per cent. in their body weight, the basal metabolism was 18 per cent. below normal. They could maintain themselves at this reduced weight on a diet of 1950 Calories and could carry out the same work as before on a diet of 2300 Calories. With further deprivation, when the body weight has been reduced by 20 per cent., there may be a return of the basal metabolic rate up to or even above the normal level.

Starvation. During starvation the whole energy expenditure is derived from the animal's own tissues. It must be remembered that the tissues of an animal comprise two distinct classes. In the first class must be placed the living substance of the body, largely composed of proteins, and in the second class are the fatty tissues, and, in a well-fed animal, a certain reserve

* Zuntz and Loewi. *Biochem. Ztschr.*, **1918**, **90**, **244**.

† Benedict *et al.* *Carnegie Inst.*, *Publ. No.* **280**, **1919**.

of glycogen, deposited in the liver and the muscles, which function simply as a storehouse of material which can be utilised for the production of energy. The total amount of glycogen present at any time is generally so small in comparison with the fat of the body that it cannot provide the energy necessary for the maintenance of life during prolonged inanition, although it plays an important part during the first one or two days.

According to the evidence of men who have been deprived of food for considerable periods, complete deprivation of food is not painful, provided water is available. During the first day or two there is a craving for food at meal-times. This often passes off, and during the later portions of the experiment even the desire for food may be entirely absent. The essential characteristic of the state of inanition is an ever-increasing weakness, accompanied by a strong disinclination to undertake any mental or physical exertion. The animal passes its time in a state of sleep or semi-stupor. Especially instructive in this connection are the cases of the so-called professional 'fasting-men,' two of whom, Succi and Cetti, have been subjected to complete metabolic investigation during their starvation. In the case of Succi, who fasted for thirty days, considerable muscular exertion was, by an effort of will, undertaken on the twelfth and on the twenty-third day of starvation without any appreciable ill-effects. The pulse rate and the body temperature remain nearly normal until a few days before death, which is ushered in by an increase in the somnolent condition of the animal and by a gradual slowing of respiration and fall of temperature. The urine is naturally diminished, with diminution in the output of urea and in the amount of water consumed. Some fæces are formed, and may be voided during or at the close of the starvation period. In Succi their amount varied from 9·5 to 22 grammes a day and contained from 0·3 to 1·0 gramme nitrogen per day.

During the whole of the starvation period there is a steady loss of body weight. In man, the daily loss of weight during the first ten days amounts to between 1 and 1·5 per cent. of the original total weight. This loss of weight does not affect all parts of the body alike. It might be imagined that, since the loss of weight is determined by the using up of the tissues of the body for the production of energy, those organs which are most active should show also the greatest loss of weight. The very reverse of this is the case, as will be seen from the following Table :

PERCENTAGE LOSS OF WEIGHT OF DIFFERENT ORGANS AND TISSUES DURING STARVATION (VOIT)

Tissue	Percentage loss of weight of fresh tissue	Percentage loss of dry tissue.
Fat	97	—
Spleen	67	63
Liver	54	57
Testes	40	—
Muscles	31	30
Blood	27	18
Kidneys	26	21
Skin and hairs	21	—
Intestine	18	—
Lungs	18	19
Pancreas	17	—
Heart	3	—
Brain and spinal cord	3	0

Those organs of the body which are most necessary for the maintenance of life, the brain, the heart, the respiratory muscles such as the diaphragm, undergo very little loss of weight. Of the other tissues the fat, which is a mere reserve to provide for such contingencies, is drawn upon first, and during starvation 97 per cent. of the total fat of the body may be consumed. The nitrogen needs of the body during starvation seem to be supplied chiefly at the expense of the muscles and glands. Hence it is easy to understand the condition of muscular inactivity which characterises the state of inanition. We may suppose that during starvation, all tissues undergo a process of slow autolysis, giving up the products of this process to the blood. The nutritional demands of a tissue being determined by its activity, the active tissues take up the material set free from all the other cells of the body and so maintain their weight at the expense of all other parts. A similar predominance of the nutrition of active over inactive tissues is to be observed in cases of partial starvation, *i.e.* where the deprivation of food applies only to a single food constituent. Thus Voit fed pigeons on a food which, while normal in all other respects, contained a deficiency of calcium salts. After a certain length of time, it was found that while the bones used in the necessary movements of the animals presented a normal appearance, the others, such as the sternum and skull, showed a marked deficiency of lime salts and had undergone a process of rarefaction giving rise to the condition known as osteoporosis. Many other instances of the sacrifice of a less essential tissue on behalf of tissue of high physiological value are known. Thus the salmon lays its eggs in the fresh water of the upper reaches of rapid streams. An adult salmon leaves the sea in the early summer months in a magnificent state of muscular development, fit to perform the prodigious feats of swimming which are required in order to get it over the rapids of the river which it has to ascend. It takes no food. In the upper reaches of the stream or river there is a growth of the ovaries or testes. The whole material for the growth of these large organs is derived from the atrophy of the skeletal muscles. In this case we have the growth of an active tissue at the cost of an inactive one, the activity, however, being determined by what we may speak of as the 'physiological habit' of the animal.

The animal organism, in the complete absence of food, deals with the resources of its tissues with the utmost possible economy. The total metabolism therefore sinks rapidly during the first two days of starvation, and then more slowly. There is indeed a slight continuous diminution with the fall in body weight, but the total metabolism per kilo. body weight till within a day or two before death is not changed so much. This is shown in the following Table of the output of energy in man during a five days' period of starvation (Tigerstedt):

METABOLISM DURING STARVATION (MAN)

Day of experiment.	Nitrogen output.	Fat oxidised.	Total Calories	Calories per kilo body weight.
1	12·16	204·8	2231	33·3
2	12·85	190·3	2112	32·1
3	13·62	179·9	2032	31·3
4	13·67	176·4	2003	31·3
5	11·44	180·0	1979	31·4

The Metabolism of Carbohydrate, Fat, and Protein during Starvation. During the first phase of inanition, in which the body passes

from the well-fed to the fasting state, the carbohydrate stores in the body rapidly diminish. This stage lasts for one or two days at most, after which energy is derived mainly (80–90 per cent.) from fat and slightly (10–20 per cent.) from protein. In the final stages, as fat becomes scarce, the energy is derived to an increasing extent from oxidation of protein.

The relative parts played by protein, carbohydrate and fat respectively in the chemical exchanges of a starving animal may be determined in the following way: The amount of protein utilised is given by estimating the total nitrogen of the excreta by Kjeldahl's method and multiplying the result by the factor 6 25. The oxygen intake and carbon dioxide output by the lungs must also be determined. Now we know that for each gramme of urinary nitrogen there has been used 5·923 l. of oxygen to oxidise the protein, and 4·754 l. of CO_2 have been produced thereby. The amounts of oxygen and CO_2 which represent protein combustion can thus be calculated, and these quantities are now deducted from the total exchanges, and the remainders expressed as a quotient, $\frac{CO_2}{O_2}$ and called the *non-protein respiratory quotient.* By a simple calculation, the amounts of carbohydrate and fat burned can then be found; more usually a table is used, such as the following:

VALUE OF 1 L. OF OXYGEN IN NON-PROTEIN RESPIRATORY QUOTIENT
(After McCLENDON)*

One litre of oxygen is equivalent to

Non-protein R Q	Grams		Calories
	Carbohydrate	Fat	
1 00	1 232	0 000	5 047
0 95	1 010	0 091	4 985
0 90	0 793	0 180	4 924
0 85	0 580	0 267	4 862
0 80	0 375	0 350	4 801
0 75	0 173	0 433	4 739
0 707	0 000	0 502	4 686

For example, suppose that a man excretes 10 grammes N in twenty-four hours, uses 426 l. of oxygen, and produces 322 5 l. of CO_2.

Then the oxygen and CO_2 corresponding to the nitrogen output would be 59 23 l. and 47 54 l. respectively. Deducting these from the respiratory exchanges we have a non-protein exchange of:

$$322\ 5 - 47{\cdot}5 = 275 \text{ l. } CO_2 \text{ and } 426 - 59 = 367 \text{ l. } O_2$$

with a non-protein R.Q. of $\frac{275}{367} = 0{\cdot}75$

and for this each l. of oxygen = 0·173 grammes carbohydrate and 0 433 grammes fat. Hence the material used is:

$10 \times 6{\cdot}25$ g. = 62 5 g. protein,	which = $62\ 5 \times 4\ 1$ =	256 Cal.
$367 \times 0{\cdot}173$ g. = 63·5 g. carbohydrate,	which = $63\ 5 \times 4\ 1$ =	260 Cal.
$367 \times 0\ 433$ g. = 159 g. fat,	which = $159 \times 9\ 3$ =	1480 Cal.
	Total Calories =	1996

If, as we suppose, the subject is fasting, the carbohydrate used would be glycogen. A check on this calculation is furnished if the individual can be placed at the same time in a calorimeter.

* McCLENDON and MEDES. "Physical Chemistry in Biology and Medicine."

The influence of glycogen metabolism on that of protein during the first and second days of fasting is shown in the experiments given below :

	First day.			Second day		
	Glycogen metabolised		N. eliminated	Glycogen metabolised		N eliminated.
	Total.	Per kilo		Total.	Per kilo.	
S.A.B.	181·6	3·15	5·84	29·7	0·52	11·04
S.A.B.	135·3	2·31	10·29	18 1	0·31	11·97
S.A.B.	64·9	1·09	12·24	23·1	0·39	12·45
H.C.K.	165·6	2·33	9·39	44·7	0·64	14·36
H.R.D.	32·8	0·59	13·25	41·6	0·76	13·53

The relative part taken in the production of the total energy by fats and proteins respectively may vary according to the amount of fat available in the body. If the subject previous to the experiment has been receiving a diet rich in protein, the excretion of nitrogen and urea in the urine diminishes rapidly during the first days of starvation. During the first two

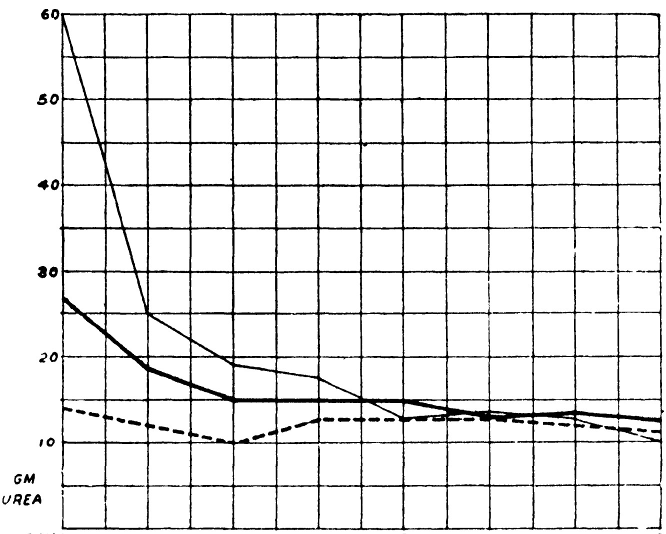

FIG. 514. Three Experiments on the Output of Urea during Starvation (dog). (TIGERSTEDT, after VOIT.)

In (1) (thin line), the dog received 2500 grammes meat per day before the experiment; in (2) (thick line), the diet was 1500 grammes meat; and in the third experiment (dotted line), the meat was reduced to a minimum.

days, therefore, a considerable proportion of the necessary energy is obtained at the expense of protein. Between the third and fifth day the nitrogenous excretion reaches a minimum, at which point it remains approximately constant until a day or two before death. If the previous diet was very poor in protein, the excretion of nitrogen may be low throughout the whole course of the experiment. These facts are illustrated by the curves in Fig. 514, which show the output of urea in three experiments on a dog on high medium and low previous diets of protein. These experiments

indicate that it is not possible, by giving extra protein in the diet, to provide any large store of body protein. The potassium excretion in starvation runs parallel to that of nitrogen, and corresponds to the potassium content of the lost muscular tissue.*

In prolonged fasting the nitrogen output falls gradually to a very low level as is shown below.

DAILY NITROGEN EXCRETION OF SUCCI IN STARVATION

Day.	N.	Day.	N.	Day.	N
1	17·0	8	9·74	15	5 05
2	11·2	9	10·05	16	4·32
3	10·55	10	7·12	17	5·4
4	10·8	11	6·23	18	3·6
5	11·19	12	6·84	19	5·7
6	11·01	13	5·14	20	3·3
7	8·79	14	4·66	21	2·82

Often just before death there is a rise of protein metabolism, synchronous with a practically complete disappearance of fat from the body. The animal now has to supply all its requirements at the expense of protein, which accounts for the increased excretion of nitrogen. This is shown in the following experiment of Rubner on a rabbit:

Days	Average daily output of nitrogen.	Average amount of fat oxidised daily.
1–3	1·67 grammes	10·3 grammes
4–5	1·46 ,,	10·3 ,,
6–8	3·21 ,,	2·4 ,,

We see therefore that during starvation, apart from the first day or two, the animal derives the main portion of its necessary energy from the combustion of fats, provided that there is a sufficient store of these substances in the body. Since protein comes from the working tissues of the body, these are spared so far as possible, and it is only when the stored fat is used up that any large call is made on the tissue protein.

It is probable that part at least of the protein that becomes available in food deprivation comes from the liver. Some also comes from blood plasma, which shows a fall in serum albumin. Hunger œdema, which is especially associated with chronic protein shortage, appears to be a result of this.

In starvation the ability to metabolise carbohydrate is depressed, so that if glucose is administered, there is an abnormally high blood sugar, and glucose is excreted in the urine—so-called hunger diabetes.

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4. EFFECT OF THE FOODSTUFFS ON METABOLISM

Food not only supplies energy, but also serves to replace the wear and tear of the body. So far as the latter function is concerned the proteins of the food take a position apart. Every working cell of the body consists largely of proteins; to repair the wear and tear of the machine of the body, therefore, proteins are absolutely necessary. To supply the energy requirements any or all of the three classes of foodstuffs can be utilised.

* GAMBLE, ROSS and TISDALL. *J. Biol. Chem.*, 1923, **57**, 633.

The Influence of Proteins. A starving man is putting out energy and is diminishing in weight as a result. In the example cited on p. 830 the man was losing about 13 grammes of nitrogen a day and was putting out about 2000 Calories. These Calories came partly from the combustion of protein and partly from the oxidation of the fat. It would manifestly be useless to try to stay the loss from the body by giving the man 13 grammes of nitrogen in the food in the form of protein, since this would give him only 320 Calories towards his basal requirements of 2000. We should find in fact that the effect of such an administration would be practically to double the output of nitrogen from the body and to increase slightly the total output of energy. In the dog, it would be possible to stop the diminution of the body weight and the waste of tissues by giving an amount of protein equal to five times the loss during starvation. In man, such an amount would be too great for his assimilating powers and it would be necessary to give fats and carbohydrates in addition to the protein.

NITROGENOUS EQUILIBRIUM. A subject is said to be in a state of nitrogenous equilibrium when the amount of nitrogen excreted during a given period is equal to that ingested. The protein taken in with the food has a twofold function to perform. Activity of the living tissues is associated with inevitable loss of nitrogenous materials, and one of the functions of protein food is the replacement of tissue which has been lost in this way. When, however, we are feeding an animal on a pure protein diet, by far the larger portion of the food is utilised for meeting the energy requirements of the body. In this function protein presents no apparent advantages over the other two classes of foodstuffs. Its value to the animal, represented numerically by its physiological heat value as 4·1, is equivalent to the value of the same weight of carbohydrate and is far inferior to the value of fat with its heat equivalent of 9·3. If, instead of giving to the starving animal a pure protein diet, we administer a mixed diet containing also fat or carbohydrate, or both substances, sufficient to meet the normal energy requirements of the body, we can restrict the utilisation of protein more nearly to the replacement of tissue waste in the body, and are therefore able to attain nitrogenous equilibrium with a much smaller proportion of protein than is possible when this substance furnishes the whole diet. In omnivora, such as man, it is easy to attain nitrogenous equilibrium on a mixed diet with a smaller nitrogen turnover than is found during starvation. In the experiment given on p. 830 the average nitrogen output during fasting was about 13 g., and in prolonged starvation it may fall to 5 g. per day or less.

Chittenden * has shown that in man normal nutrition may be maintained on a mixed diet containing only 7 grammes of nitrogen daily. If the amount of fat and carbohydrate be very largely increased, it is possible to maintain nitrogenous equilibrium on even smaller quantities of protein. Thus Kon and Klein † kept a man and a woman, each of about 63 kilogrammes body weight, for 167 days in good health and nitrogenous equilibrium on an average daily nitrogen intake of 5·7 and 3·8 grammes respectively.

Most of the experiments on the influence of variations in the quantity of protein food have been made on carnivora, such as the dog and cat. Within very wide limits, the output of nitrogen is proportional to the intake. This is shown in the Tables by Voit given on p. 835, representing two experiments on dogs.

In Experiment I. the animal had been fed for some days before with 500 grammes of meat per diem. The fact that he was excreting nitrogen corresponding to 547 grammes of

* CHITTENDEN. "Physiological Economy in Nutrition" (New York), 1905.

† KON and KLEIN. *Bioch. Journ.*, 1928, **22**, 258.

flesh shows that this amount was insufficient and that he was not yet in a condition of nitrogenous equilibrium. Each day he was using up 47 grammes of the muscular tissues of his body, in addition to the 500 grammes supplied in the food. On increasing his food threefold to 1500 grammes the nitrogenous output was also increased, but a state of nitrogenous equilibrium was not reached until the eighth day of the experiment. During the six days intervening 778 grammes of meat had been retained in the body, *i.e.* there had been a retention of protein, probably in the form of increased muscular substance.

In Experiment II. the diminution of the protein of the food was followed by a loss of protein from the body, the output being greater than the income. The excess, however, was rapidly diminishing and equilibrium had been practically attained on the last day of the experiment. During this time the animal had excreted 14 8 grammes of

Experiment I.			Experiment II.		
Day.	Daily meat ration	Flesh loss per day	Day.	Daily meat ration.	Flesh loss per day.
1	500	547	1	1500	1500
2	1500	1222	2	1000	1153
3	1500	1310	3	1000	1086
4	1500	1390	4	1000	1088
5	1500	1410	5	1000	1080
6	1500	1440	6	1000	1027
7	1500	1450			
8	1500	1500			

nitrogen more than it had received in its food, which would correspond to a diminution in its body of 434 grammes muscular substance. Many such experiments have been performed, and they all agree in showing that in carnivora an appreciable storage of nitrogen can take place in the body, probably by a growth of muscle substance. However much protein is given, the limit is finally arrived at where no further laying on of protein tissues of the body is possible, and the animal then enters into a state of nitrogenous equilibrium. This equivalence of income and output signifies that the *total energy output* of the body rises and falls with the quantity of protein in the food. This is shown in the following Table by Pettenkofer and Voit, in which the figures have been recalculated by Pflüger:

Period.	Nitrogen in food	Nitrogen output	Fat gain or loss	Total Calories.
1	0	5·61	− 98	1067
2	17	20 37	− 61	1106
3	34	36·69	− 43	1360
4	51	51·00	− 24	1552
5	61	59·74	− 36	1893
6	68	69·50	+ 8	1741
7	85	85·41	+ 4	2181

We see, therefore, that *carnivorous animals* can satisfy their total energy requirements at the expense of protein. When the protein income is in excess of their requirements, a small amount is laid on, probably as increased muscular tissue. The most marked effect, however, is an increased metabolism, which rises in proportion to the nitrogenous income. The limit to this increase is set by the powers of the alimentary canal to digest the protein. The rise in metabolism consequent on protein food is very rapid and affects the gaseous exchanges as well as the output of nitrogen. Magnus Levy and Falk found that a large protein meal might increase the respiratory exchange 40 per cent., during seven hours. The nitrogenous output also rises immediately after a protein meal, so that 50 per cent. of the nitrogen of the ingesta may appear in the urine within seven hours after the meal.

The whole of these results cannot be strictly applied to man, in whom it is impossible to supply all the energy requirements of the body on a pure protein diet. Even if a man eats as much meat as he can, he will be unable to obtain sufficient energy for his daily needs. Whereas the average daily requirements of a man amount to about 3000 Calories, 1 lb. of lean meat (*i.e.* entirely devoid of fat) would yield only about 400 Calories, and even if he took 4 lb. of meat daily, an amount which is impossible for most individuals, he would be obtaining only about 1600 Calories. The cures for obesity, in which a large protein diet plays an important part, are in all cases practically equivalent to a state of semi-starvation.

Many experiments have been made on the influence of variations in the quantity of protein in a mixed diet. Within wide limits the output of nitrogen is strictly proportional to the intake. A normal adult man seems able to store little, if any, protein. The only way in which protein can be laid on in the body is by furnishing a physiological stimulus to the growth of muscle, *i.e.* by constant exercise. Without this it is not possible to produce growth of the muscles of the body, however much protein we may give. The conditions, however, are different when dealing with an individual in whom from some cause or other the muscular tissues have not attained their full development. Thus in growing individuals a certain amount of the protein of the food is always retained in the body and laid on as tissue protein. Weight for weight, a boy of sixteen to eighteen requires about 50 per cent. more protein than an adult man (Berg). In convalescence after great wasting of the muscles has taken place, forced feeding with large amounts of protein has been found to give rise to a considerable retention of protein in the body. This process goes on only until the muscles have attained their normal condition of development. When the tissues have, so to speak, reached 'par,' the possibility of laying on protein tissues ceases. On the other hand, protein food has in man, as in animals, a specific stimulating effect on the fasting metabolism, so that the respiratory exchange is largely increased as a result of a heavy protein meal. The rise is about 30 per cent. of the basal value; *i.e.*, if for each 100 Calories basal metabolism we give meat equal to 100 Calories, then the metabolism, instead of remaining at 100, rises to 130 Calories. This effect has been named by Rubner the '*specific dynamic effect*' of protein.

The Influence of Fats and Carbohydrates. If either fats or carbohydrates be given to a starving animal, a certain sparing of the fat of the body takes place, but this effect is accompanied by a distinct specific dynamic action, viz. from a basal value of 100 Calories the metabolism is increased to 106 Calories by 100 Calories glucose, or to 104 by 100 Calories fat. In under-nourished individuals the effects are greater than these, and tend to bring the metabolism up to what it would be if the basal level were normal. As regards the protein metabolism, Cathcart finds that while administration of fat increases the nitrogen output during starvation, carbohydrate food causes a diminution in the nitrogen output, and thus exercises a marked sparing effect on the proteins of the body.

The stimulating influence of carbohydrate on metabolism, although much less than that observed on the administration of large doses of protein, is easily demonstrable after administration of moderate amounts of soluble carbohydrate to a fasting subject. If carbohydrate be given in excess of the daily energy requirements, the greater proportion of it remains in the body, being stored up provisionally as glycogen, but eventually in the form of fat.

Specific Dynamic Action. We have seen that the specific dynamic

action of the various foodstuffs results in the augmentation of the basal rate of metabolism by the following percentage amounts :—

Protein . . .	30 per cent.
Carbohydrate . .	6 „
Fat	4 „

The augmentation is not so constant as these figures suggest, however. With protein it varies according to the amount given, first decreasing, then reaching a level and finally increasing as the total caloric contribution from protein is raised.

The explanation of specific dynamic action is not entirely clear and the effect is probably due to various causes. Since protein has the most striking effect it has received most attention. The effect is also produced by some of the amino-acids, has its seat mainly in the liver, and seems at least in part to represent heat set free in the conversion of the amino-acids to urea. It is abolished by removal of the liver.

Another view, due to Schaeffer and Le Breton, is that the specific dynamic action of protein, or of amino acids, is due to a stimulating effect of amino acids, circulating in the blood, on the secretion of adrenaline by the supra-renals. Adrenaline exerts a stimulating action on the metabolism, probably by facilitating certain oxidative changes in the tissues, principally in the liver.

The effect of carbohydrate probably represents the energy expenditure needed to convert it into glycogen. It is not abolished by liver removal.

The effect of fat is unexplained, and is supposed to be due to increased fat combustion.

The specific dynamic action of protein results only in the production of heat, and since in cold surroundings a large proportion of the food must always be applied to maintain the body temperature, the excess heat involved in the consumption of protein, and to a less extent of other foods, can be, and is, utilised. Hence it comes about that in order to exhibit this specific dynamic action, the animal must have a temperature above 33° Centigrade. Below this temperature the specific dynamic action of the foodstuffs becomes less and less apparent, and is finally merged in the heat production necessary, whether food is taken or not, to keep up the temperature of the body.

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THE EFFECT OF MUSCULAR WORK ON METABOLISM

When an animal performs muscular work, the energy for the work is immediately derived from the oxidation of glycogen, but ultimately of the foodstuffs. It results in increased intake of oxygen and output of CO_2 and in deeper and more rapid breathing. In order to determine the quantitative relationship between the increased oxidative processes and the work accomplished, we must investigate the respiratory exchanges of an individual by

one of the methods described on p. 818, in the first place during complete rest, in the second place while doing measured work. The following Table (Benedict and Cathcart) represents the respiratory exchanges in a trained muscular subject during complete rest and while riding a stationary bicycle.

Condition.	c c CO_2 eliminated per minute.	c c. Oxygen absorbed per minute.	Respiratory quotient.	Pulse rate.	Respiration. Rate.
Lying . .	200	243	0·83	56	20
Moderate work.	1720	1834	0·94	150	32
Severe work .	3227	3265	0·98	166	38

The 'severe work' in this Table was carried to exhaustion, so that the respiratory exchanges represent the maximal for that type of exercise.

We can determine the relation between work done and energy expended by either direct or indirect colorimetry, as explained on p. 825. In the Table below are given the results of an experiment by Atwater by the direct method. The work done by the man, on a bicycle ergometer, is given in the Table in terms of its heat equivalent.

In these experiments, the mean expenditure of energy on the work days was 5120, and on the rest days (with food) 2397 Calories, a difference

ENERGY PER DAY.

Nature of experiment.	Heat eliminated.			External work in Calories.	Total in Calories.
	By radiation and conduction.	In urine and fæces	In water vaporised from lungs and skin		
Rest with food (average of four days) . . .	1850	26	521	—	2397
Rest, fasting (four experiments) (average of five days)	1605	21	561	—	2187
Work (fourteen experiments) (average of forty-six days)	3802	29	743	546	5120

of 2723 Calories. This represents the *excess metabolism* over resting requirements, which must take place in order that the body may perform work equal to 546 Calories. The fraction $\frac{\text{work}}{\text{energy of excess metabolism}}$ is spoken of as the 'mechanical efficiency' of the body. In the above case it equals one-fifth, and the body is said to have a 20 per cent. efficiency. Of course a certain proportion of this excess of energy used over work done is accounted for by the increase in the work which must be performed by the respiratory muscles and heart. Even if we neglect these factors altogether, the efficiency of the body as a machine corresponds to between 16 and 25 per cent., an efficiency which exceeds that of the best of our steam engines and is only equalled by certain internal combustion engines.

We have already seen in Book II that the results of metabolism experiments during work may throw light on the nature of the constituents of the body which undergo oxidation to furnish the energy of muscular work.

In the experiment already quoted, the oxidation of carbon was increased more than threefold during a six-hour period of work. This carbon might form part of the molecule of protein, fat or carbohydrate; but it has long been known that muscular exercise causes no considerable increase in the nitrogen excretion.* The above experiments illustrate this fact. The basal output of nitrogen per day corresponded to the destruction of 82 grammes of protein. During rest and with food, the average daily consumption of protein was 98·8 grammes. During a work day, in which the individual received the same amount of protein and a somewhat insufficient quantity of carbohydrates and fats, the consumption of protein was 109·4 grammes. Thus there was a three- to four-fold increase of the carbon metabolism of the body, but only a 10 per cent. increase in the protein metabolism.

The study of the respiratory quotient, if derived from observations over periods of several hours, might be expected to give us some idea of the nature of the material which is furnishing by its oxidation the necessary energy for the performance of muscular work. If the whole of the animal's energy requirements were furnished by the oxidation of carbohydrates, the output of carbon dioxide expired would be exactly equal in volume to the oxygen taken in. Thus: $C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2$, and the *respiratory quotient* of the animal, namely $\frac{CO_2 \text{ expired}}{O_2 \text{ inspired}}$, would be equal to unity. If we compare the formulæ of a carbohydrate and a fat respectively, it is evident that it will require a relatively larger amount of oxygen to oxidise the fat than is necessary in the case of the carbohydrate. In an animal burning only fats, the carbon dioxide output of the body would be considerably less than the oxygen intake and its respiratory quotient would be less than unity. The respiratory quotients for protein, fats and carbohydrates are given in the following Table:

Material.	Respiratory quotient $\frac{CO_2}{O_2}$ (volumes)
Carbohydrate	1·0
Protein	0·81
Human fat	0·718

Since the carbon dioxide excreted during exercise may result from the oxidation of protein, fat or carbohydrate, we cannot directly deduce the total energy set free in the body by an estimation of the O_2 intake alone. The respiratory quotient must be determined, in order to throw light on the real amounts of fat and carbohydrate consumed, and the output of nitrogen in the urine must also be measured. Then the non-protein R.Q. is determined as already explained on p. 831, and from this the relative contribution of the various foodstuffs can be deduced by reference to a table such as the one following, which gives the relative oxygen usage for combustion of carbohydrate and fat for a given non-protein respiratory quotient in man †:

R.Q.	Carbohydrate.	Fat.	Calories for one litre oxygen.
1·00	100 per cent.	0 per cent.	5·047
0·95	82·3 ,,	17·7 ,,	4·992
0·90	65·5 ,,	35·5 ,,	4·936
0·85	46·8 ,,	53·2 ,,	4·881
0·80	20·1 ,,	70·9 ,,	4·827
0·75	11·4 ,,	88·7 ,,	4·770
0·718	0 ,,	100 ,,	4·735

* Fick and Wislicenus. *Vierteljahrs. d. zürch. Naturf. Gesselsch.*, 1865, **10**, 317.

† Cathcart and Cuthbertson. *Journ. Physiol.*, 1931, **72**, 349. The slightly different values from those on p. 831 is due to the fact that the composition here taken for fat is that of human fat.

The number of Calories set free in the body for every litre of oxygen taken in are also given in the above Table.

The respiratory quotient in an animal is therefore a net result determined by the average nature of the substances which are undergoing oxidation in its body. If the performance of muscular work involved special chemical processes, *e.g.* a metabolism of one of the main constituents of the body in preference to either of the others, this sudden change in the quality of the metabolism should show itself in the respiratory quotient. It must be borne in mind that the respiratory quotient gives only the final result of the oxidative changes, and tells us nothing regarding the intermediate stages by which that end result is reached. For instance, if fat were converted to carbohydrate and the carbohydrate all burned, the respiratory quotient would be the same as if the fat had been burned directly.

Prolonged moderate exercise, although attended by a large increase in the CO_2 output and oxygen intake of the body, does not alter the respiratory quotient.*. The respiratory quotient over a long period, such as a day, must depend on the material which is combusted, irrespective of the amount of muscular work which is done in the time. Ultimately there is no doubt that the energy for muscular exercise can be derived from any of the three classes of food, carbohydrates, fats and proteins. Such experiments, however, do not answer the question as to the immediate origin of the energy involved in muscular contraction. We shall see later on that the body has the power of converting carbohydrates into fats; and proteins, and perhaps fats, into carbohydrates. The respiratory quotient in the course of a day is affected by these intermediate changes as well as by the oxidised foodstuffs. When dealing with muscular contraction we saw that the ultimate source of the energy of contraction was the glycogen of the muscle.

As the result of a series of experiments Krogh and Lindhard † came to the conclusion that the cost of work in man, when it is performed at the ultimate expense of fat, is 10 per cent. more than when it is performed at the expense of the carbohydrate of the body. This extra expenditure of energy they regard as, so to speak, wasted in the chemical conversion of fats into carbohydrates. This great difference does not arise in the case of proteins, which form carbohydrates in the body with extreme ease. But whereas proteins form, for the most part, the working tissues of the body, fats are stored as a reserve food, so that under normal conditions, in default of an excessive diet of proteins, fats must often be utilised for the carrying out of muscular work. For this to take place, it is probable that they are first converted into carbohydrates and stored in the muscles as glycogen.

It will be noticed that, in contradiction to popular opinion, the proteins do not play any special part in relation to muscular exercise.‡ The special value of protein is for building up the body and for repairing wear and tear. As a source of energy it presents no advantages over carbohydrate and fat; in fact it may be in some respects inferior to these substances, or at any rate less economical, owing to the specific dynamic action, the whole of which appears in the form of heat. If an animal after a protein meal be made to work, the increased metabolism due to the specific dynamic action of the protein is still observed over and above that due to the performance of work. On the other hand, in training there is some reason for a plentiful supply of protein in the diet. When, however, a man has arrived at the full development of his muscular system, there is no need to attempt to supply the energy for his movements at the expense of protein. In many cases the beneficial effects of so-called meat are due to its content of fat.

* Gemmill. *Amer. J. Phys.*, 1931, **98**, 135.
† Krogh and Lindhard. *Bioch. J.*, 1920, **14**, 290.
‡ Wilson. *J. Physiol.*, 1932, **75**, 67.

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5. THE SIGNIFICANCE OF THE FOODSTUFFS

ISODYNAMIC REPLACEMENT. The actual amount of energy required as food will depend on the size of the animal, on the temperature of the surrounding medium and on the muscular work performed. For supplying this energy any of the three classes of foodstuffs may be utilised, the value of each being given by the Calories evolved when the foodstuff is oxidised to the end stages which it attains in the body. Thus carbohydrates and proteins are *isodynamic—i.e.* give rise to the same amount of energy in the body for a unit weight—1 gramme of each giving out 4·1 Calories. Within limits carbohydrate can be replaced by the isodynamic amount of fat, and within narrower limits all three classes are isodynamically interchangeable. This isodynamic equivalence of the foodstuffs is, however, interfered with to a certain extent by the specific dynamic action. On account of this specific dynamic action, an average mixed diet, in order to maintain the body in a state of equilibrium, must have a Calorie value of about 15 per cent. higher than the basal requirement. Thus, supposing a man while fasting and in bed gives off 1800 Calories a day, he would under the same circumstances give off about 2000 Calories if he received a minimum mixed diet just sufficient to maintain his body weight constant.

PROTEIN

Proteins are distinguished from carbohydrates and fats by the fact that no means are provided for the storage of proteins in the body, except by the growth of muscular and other tissues, and perhaps, to a certain extent, by actual storage of protein in the liver.* Arrived at its maximum state of growth, the body cannot add to its protein store, and therefore all the protein that is ingested and absorbed is at once broken down in the body. If we examine the curve of nitrogenous excretion in the urine, we find that practically all the nitrogen taken in as protein in the food is turned out within twelve to sixteen hours. Thus, if a man is on a mixed diet sufficient for his daily needs, a doubling of the protein in the diet, leaving the other constituents unchanged, will result in a doubling of the nitrogenous excretion. On the other hand, an increase of the fats or carbohydrates will not increase the carbon output of the body, but any excess above the daily needs, if absorbed, is stored up in the body in the form of fat. To increase the protein metabolism therefore, all that is necessary is to increase the protein intake; to increase the carbohydrate and fat metabolism it is necessary to increase the metabolism as a whole, e.g., by muscular exercise or by exposure to cold.

Protein is essential for the repair of the wear and tear of the tissues. We may thus speak of the protein of the food as having a twofold destiny. The amino-acids into which it is resolved circulate in the blood, and each living cell picks out those amino-acids which are essential for building up the

* FOSTER and WHIPPLE. *Amer. J. Physiol.*, 1922, **58**, 393, 407; STÜBEL, *Pflüger's Arch.*, 1920, **185**, 74.

protoplasm of the cell. The rest of the protein, after resolution into amino-acids, loses its nitrogen, which appears in the urine chiefly in the form of urea, and the remaining part of the protein molecule ultimately undergoes oxidation. The nitrogen of the urine is therefore derived, partly *exogenously*, *i.e.* direct from the nitrogen split off from the protein of the food, partly *endogenously*, *i.e.* from the disintegrated proteins which have formed part of the living cells.

Folin brought forward a number of facts, which suggest that the nitrogenous end products of nutritional metabolism are different from those of the energy metabolism. There is also a difference in the time relations of the two orders of metabolism. Whereas the nitrogen is rapidly eliminated when protein is being utilised for the supply of energy to the body, the occurrence of increased tissue waste causes a rise of nitrogenous excretion, which comes on slowly, often after the lapse of a day, and may last two or three days. These aspects of the subject will be dealt with in Chapter XLVI.

ACTION OF THE PRODUCTS OF PROTEIN DIGESTION. Since the proteins of the body differ in their composition from the majority of the proteins of the food, it is evident that each molecule has to be entirely disintegrated and reconstructed before it can take its place in the body fabric; and it is therefore only natural that, so far as metabolism is concerned, the results should be identical whether we feed the animal with the ordinary food-protein or with the products of its hydrolysis. A mixture containing only amino-acids was formerly thought to be inadequate as compared with a protein digest; this was because the amino-acids were not then all known; we now know that if all the essential amino-acids, including threonine, are used, the mixture has the same nutritive value as protein, and can be given either by mouth or by slow intravenous injection.

The Biological Value of Different Proteins

In consequence of the variation in the composition of the proteins contained in the different tissues of the body, it is necessary that the food shall include certain of the amino-acids in proper quantities. The indispensable amino-acids, ten in number, are called *essential*; the remainder can be synthesised in the body and are hence *non-essential*. Hence it follows that all proteins are not equivalent as regards their capacity for replacing tissue waste or serving for growth. Those in which nearly all the amino-acids are represented and which contain these in proportions approximating the average of those found in the chief tissue proteins are called *first-class proteins*. Most animal proteins belong to this group, though gelatin is an exception; some vegetable proteins such as edestin, glutenin of wheat, or glutelin of maize, are also included.

In the remaining, or second-class proteins, either (1) one or more of the essential amino-acids are absent, or (2) there is a large preponderance of one or more of the amino-acids which are required only in small proportions. As an example of the first type we may instance collagen and gelatin, and zein, the chief protein of maize. As an example of the second type we may instance the protein of wheat flour. Whereas caseinogen contains 22 per cent. of glutamic acid and serum albumin only 8 per cent., gliadin, the chief protein of flour, contains as much as 36 per cent. If wheat flour is the main or sole source of protein to the body, it is evident that a large amount of glutamic acid will be in excess of that required. It is also deficient in lysine.

One of the methods of studying the subject is to feed young animals, *e.g.*, rats, on a diet in which the protein lacks certain amino-acids, and to

follow the rate of growth. This is reduced if the lacking amino-acid is essential. Gelatin is lacking in cystine, valine, isoleucine, tyrosine, tryptophan and hydroxyglutamic acid, and it has long been known that it cannot entirely replace the proteins of the food. Its capacity for yielding energy to the body equals that of any other protein, but it can only replace body protein when the missing amino-acids are also supplied. In the same way zein, which is deficient in lysine and tryptophan, is incapable by itself of supporting life, though this can be accomplished if tryptophan is administered at the same time. Growth does not occur, however, until lysine is also added (Fig. 515).

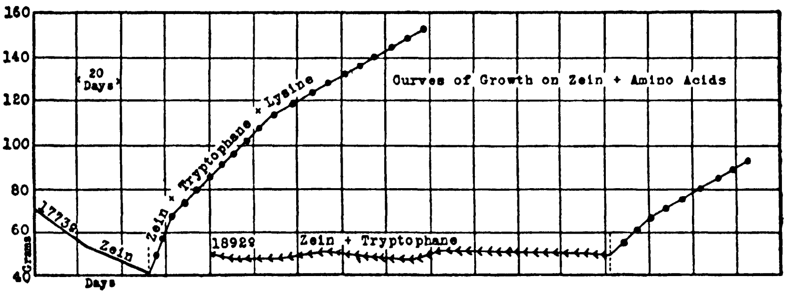

FIG. 515. Growth curves of young rats fed on a diet in which the protein is given as zein. No. 1773 received zein and lost in weight; when tryptophan and lysine were added to the zein it gained weight. No. 1892 received zein and tryptophan for six months; its weight remained stationary, but showed rapid increase when lysine was also given. (MENDEL. Courtesy of the *J. Amer. Med. Assoc*)

Certain proteins are also deficient in the sulphur-containing amino-acid methionine, some in phenyl-alanine, and some in histidine. All these, together with lysine and tryptophan, are essential, and cannot be made in the body. It is noteworthy that cystine can be replaced by methionine, and tyrosine by phenyl-alanine; but histidine cannot be replaced by arginine.

Undoubtedly the best method of finding which amino-acids are essential and which are not is to feed young animals on a diet in which protein is replaced by mixtures of pure amino-acids, one of which is omitted. This method has been used with remarkable success by Rose, and threonine was actually discovered in this way, as it was observed that mixtures of all previously known amino-acids were inadequate to promote growth. One of the consequences of lack of essential amino-acids is failure of growth; another is lack of appetite. Lack of valine produces curious rotatory movements in rats.

The following Table shows the essential and non-essential amino-acids, according to Rose.*

Essential.	*Non-essential.*
Arginine.	Alanine.
d- or *l*-Histidine.	Aspartic acid.
d-Isoleucine.	Citrulline.
l Leucine.	Cystine.
d-Lysine.	Glutamic acid.
l-Methionine	Glycine.
d- or *l*-Phenylalanine.	Hydroxyglutamic acid.
d Threonine.	Hydroxyproline.
d- or *l*-Tryptophan.	Norleucine.
d-Valine.	Proline.
	Serine.
	Tyrosine.

* ROSE. *Physiol. Rev*, 1938, **18** 109.

Nearly all the experiments on essential amino-acids have been carried out on rats. It is not known to what extent the results apply to other species.

These differences in chemical composition between the proteins are of greatest significance when the protein of the food is reduced to the minimal amount required to replace tissue waste, and under such circumstances marked differences are observed between the biological values of proteins. The biological value of a given protein decreases as the amount of that protein is increased.

It is evident that when there is scarcity of protein, animal protein is more economical than vegetable protein; and that if it is necessary to live on a purely vegetable diet this should be mixed, so that all the amino-acids required in the body may be represented in the diet.

For the estimation of the biological value of proteins in producing growth, the method of Osborne, Mendel and Ferry is used. In this the nutritive value is expressed in grammes of body weight increase per gramme of protein consumed, when the diet contains that amount of protein at which the maximal growth occurs.

Revolutionary claims regarding the minimal quantity of protein which is necessary in order to preserve nitrogenous equilibrium have been made by Berg.* He states that this depends not only on the nature of the protein, but also on certain conditions of the experiment. First, the subject will utilise a particular protein better if he has become habituated to it; if a vegetarian is given animal protein, or a meat-eater a pure vegetable diet, diarrhœa results, and there is imperfect utilisation of the protein taken in. Secondly, the minimum protein requirement on a given protein diet will vary according to the ratio of inorganic bases to inorganic acids available to or formed in the body. When the acids are in excess, so that strongly acid urine is excreted, a greater amount of protein is needed than when the bases are in excess. This is perhaps partly because, when the acids are in excess, some of the nitrogen of the protein, in the form of ammonia, must be utilised in order to neutralise them. The fixed inorganic acids of the body are to a great extent formed in the course of metabolism, *e.g.* sulphuric acid and phosphoric acid from the metabolism of proteins and nucleo-proteins. The bases are derived principally from the food, and are particularly abundant in certain kinds of vegetable foods. When a subject fed on 200 grammes of calves' thymus with 75 grammes cream daily, and took with it base-yielding foods in the form of potato and apples, his daily nitrogen requirement was about 7·2 grammes. If, next, he took the thymus with an acid-forming type of bread, instead of potatoes, the daily nitrogen requirement rose to about 11·3 grammes, *i.e.* an increase of about 57 per cent. When thymus and margarine only were taken, which would lead to considerable amount of unneutralised acids, the requirement was 23·4 grammes per day, or 225 per cent. above the initial amount. The protein requirements of children are not so much affected by excess of acids as are those of adults.

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FATS

Besides their high calorific value and their frequent content of vitamins, there is evidence that fats are essential constituents of diet. It is admitted that they are, in the form of lecithins, indispensable components of most

* BERG, "Eiweissbedarf und Mineralstoffwechsel bei Einfachster Ernährung," 1931 (Hirzel, Leipzig.)

living cells, and although certain fats are readily synthesised in the body, it is doubtless more economical for them to be supplied as such in the diet. Moreover, there seems good ground for regarding small amounts of unsaturated fatty acids such as linolenic and linoleic acids, or their esters, as being quite indispensable for proper nutrition.* Rats fed on entirely fat-free but otherwise complete diets developed a deficiency disease affecting the skin, and their growth and reproduction were impaired. Addition of small amounts of the acids or fats named effected cure, or prevented the disease.

As will be explained later when dealing with fat metabolism, a certain amount of choline is probably an essential constituent of a diet.

6. THE INORGANIC FOODSTUFFS

The Water Balance. Some 70 per cent. of the body consists of water, and the water content of the tissues is maintained reasonably constant by equalisation of the loss and gain of water.

Even when very large amounts of water are drunk there is no considerable dilution of the blood; nor is there measurable concentration of it on deprivation, except in extreme thirst.† By delicate methods, however, changes can be shown to occur.‡

The gain of water is derived from two sources, viz. water taken in as such with food or drink, and water formed by the oxidation of foodstuffs. The output of water is by the lungs, skin, urine and fæces.

The water formed by oxidation of foodstuffs can be calculated on the assumption that, from 100 grammes of each of the proximate principles, the following amounts of water are derived :

From protein	41 grammes.
,, fat	108 ,,
,, carbohydrate	55 ,,

Calculation on this basis shows that with average sedentary diets about 450 c.c. of water per day are formed by oxidation.

The total exchange of water per day is approximately as follows for average conditions for an adult :

Income.		Output.	
Drink	1,450 c.c.	Urine	1,500 c.c.
Food	800 ,,	Skin	600 ,,
Oxidation	350 ,,	Lungs	400 ,,
		Fæces	100 ,,
	2,600 c.c.		2,600 c.c.

The proportion of the water lost by the respective channels of kidneys, skin, lungs or bowel will naturally vary with conditions. In diuresis relatively more will pass out by the urine, in sweating more by the skin, when dry air is breathed more by the lungs, in diarrhœa or cholera more by the bowel; when more passes out by one channel, there will be less to be eliminated by the other routes.

* McAmis, Anderson and Mendel, *J. Biol. Chem.*, 1929, **82**, 247; Burr and Burr, *ibid.*, 1929, **82**, 345; Burr, Burr and Miller, *ibid.*, 1932, **97**, 1; Drummond and Gregory, *Ztschr. f. Vitaminforsch.*, 1932, **1**, 357; Evans and Lepkovsky, *J. Biol. Chem.*, 1933, **99**, 231.

† Haldane and Priestley. *J. Physiol.*, 1915, **50**, 296.

‡ Priestley, *J. Physiol.*, 1915, **50**, 304. Margaria. *J. Physiol.*, 1930, **70**, 417.

In a person at rest and not visibly perspiring, the loss of water by lungs and skin can be estimated by weighing the subject at intervals ; the loss of weight is mostly due to water loss, but allowance can be made for the small loss due to the difference in weight between oxygen intake and CO_2 output. Proceeding in this manner, it has been found * that the rate of loss from " insensible perspiration " is proportional to the basal metabolism, and in an average man is about 40 grammes per hour, of which 18 grammes represents water lost by the lungs, 18 grammes water lost by skin, and 4 grammes the balance between weights of O_2 intake and CO_2 loss.†

With alterations of body-weight there is usually a proportional loss of water and of solid constituents, but in some cases there is a proportionally greater loss or gain of water, which it is important to take into account. A loss greater than 20 per cent. of the body water is not usually compatible with life.

Saline Constituents. The inorganic foodstuffs comprise the mineral salts and water. The salts are essential to life. If an animal be fed with a diet from which all the salts have been removed, it loses appetite, becomes ill, and dies sooner than if completely starved. An animal in a state of salt hunger becomes very weak, and shows signs of paralysis; food, still undigested after prolonged periods in the stomach, is vomited up, and it is clear that the functions of the entire body are profoundly upset.

Foster has shown that in salt-hunger the body is continually giving off inorganic constituents in the urine. The amount of these is smallest when the animal is supplied richly with organic foodstuffs. It seems that certain inorganic ions are held in a state of combination with the tissue constituents.‡ If the amount of food supplied is insufficient, the animal lives on its own tissues, thus setting free salts which appear in the urine. An interesting condition is miner's cramp, met with in workers who drink water and perspire freely in hot mines. So much sodium chloride is lost in the sweat that the urine, though small in bulk, is sometimes quite free from chloride.§ The cramp is due to excess of water in the tissues, and can be avoided by the subject drinking water containing 10 grains of salt per gallon (0·105 per cent.)

A large number of elements are probably needed for the maintenance of health and normal growth, and these are required by the body in very different amounts. The daily adult intake for some of the more important may be put approximately at :

Calcium	0 7 g.
Sodium	6·0 ,,
Potassium	4 0 ,,
Iron	12 mg.
Phosphorus	1·25 gr.
Iodine	0 05 mg.
Chlorine	20 0 g.
Zinc	12 mg.

Most of these elements are taken in excess of the minimal requirements, any excess over this being excreted, and it is often uncertain what the minimal requirement actually is. Usually a state of balance is maintained, as in the following example for Ca, Mg and Fe :

* BENEDICT and ROOT. *Arch. Int. Med.*, 1926, **38**, 1.
† BENEDICT and BENEDICT. *Proc. Nat. Acad. Sci.*, 1927, **13**, 364.
‡ BAIRD and HALDANE. *J. Physiol.*, 1922, **56**, 259.
§ HANCOCK, WHITEHOUSE and HALDANE. *Proc. Roy. Soc.*, 1929, **105B**, 43.

BALANCE SHEET FOR CALCIUM, MAGNESIUM AND IRON.
(Amounts for six subjects over fourteen days (MCCANCE and WIDDOWSON.*)

	Intake from Food, g.	*Output, g.*			*Balance, g.*
		Urine.	*Fæces.*	*Total.*	
Calcium .	61 68	13 69	48 99	62 68	− 1·00
Magnesium	22·80	9 28	13 30	22 58	+ 0·22
Iron .	0 6033	0 0078	0·607	0·6148	− 0·0115

Sodium, chiefly in the form of chloride, plays an important part in the maintenance of the proper osmotic pressure of the body fluids, and the sodium ion is also essential in proper concentrations for the normal functioning of all cells. Sodium deficiency is rare in Europe, but is seen after removal or disease of the suprarenal cortex.

Potassium is present in most foodstuffs, and deficiency of it is very rare.

Iron is essential for the formation of hæmoglobin and of the various hæmatin-like compounds, such as cytochrome, which play a very important part in carrying on the oxidative processes in the body. Much of the iron taken by the mouth is unabsorbed. Milk is poor in iron (about 1 mg./l.), but the infant is born with a store of iron in the liver sufficient to last it for several months. Good sources of iron are meat, potatoes, liver, kidney, watercress and most vegetables. Iron compounds are probably reduced to the ferrous state in the alimentary canal, and unabsorbed iron is excreted as ferrous sulphide. Deficiency of iron leads to anæmia, but the taking of excess of iron does not lead to formation of larger amounts of hæmoglobin than normal.

Very little iron is excreted per day, the iron store of the body being kept up by conservation of iron. When the hæmoglobin of the red cells is disintegrated on their destruction, the iron is stored for a time as a ferric hydroxide complex with protein, known as *hæmosiderin*, in the liver, and is re-utilised as demand arises for the construction of fresh hæmoglobin.

Copper in a very small amount appears to be necessary for the assimilation of iron and formation of hæmoglobin.

Manganese is another element which must be supplied in minute amounts. In its absence the processes of reproduction are disturbed.

Magnesium is also essential. It is chiefly present in the body in the bones, as a phosphate. A rat requires not less than 5 mg. of Mg. per 100 g. food. The human requirement is about 0·6 g./day. In magnesium deprivation there are vascular disturbances, hyperexcitability, convulsions and death.† The effects of Mg. deficiency are intensified if the diet is very rich in Ca.‡ The Mg ion acts antagonistically to the Ca ion with regard to the excitability of tissues.

Chloride is the chief anion of the body : it is readily absorbed as soluble chlorides, which are present in excess in most European diets. In shortage of chloride the ion is tenaciously retained in the body, and the urine, which normally carries the excess of ingested chloride over the requirements, may then become totally chloride-free.

Iodide is a constituent of thyroxin, the hormone of the thyroid. About half of the body iodine is present in the thyroid. Iodides are rapidly absorbed and are excreted unchanged.

Bromide is also quickly absorbed, and is normally present in traces in the body, where it mingles with the chlorides.

* MCCANCE and WIDDOWSON. *J. Physiol.*, 1938, **94**, 148 ; *Bioch. J.*, 1939, **33**, 523.
† KRUSE, ORENT and MCCOLLUM. *J. Biol. Chem.*, 1932, **96**, 519.
‡ TUFTS and GREENBERG. *J. Biol Chem.*, 1938, **122**, 715.

Calcium * is most abundant in milk and cheese; eggs and green vegetables contain some, but meats, fish and bread are poor in calcium. Some of the calcium requirement can be obtained by drinking hard water. Much of the calcium of the diet is not absorbed, the fraction so remaining depending on the other constituents of the diet, and on the extent to which the duodenal contents retain an acid reaction. Bile salts and sugars aid in the absorption of calcium, while alkalies or large amounts of phosphates retard it by formation of the insoluble calcium phosphate. Fats also retard it. Growing children and pregnant women and nursing mothers need more calcium than other persons, and about 1·6 g./day is suggested for them.

Calcium is essential for the formation of the bones and teeth, for blood

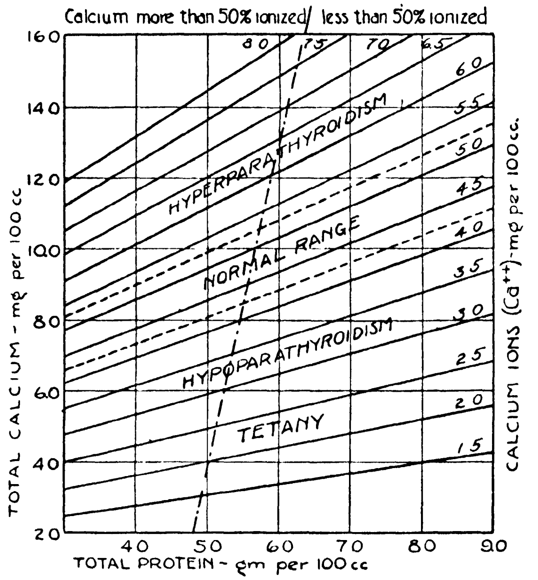

FIG. 516. Nomogram for finding Ca·· of serum from total Ca and protein. The position of the point of intersection of any pair of rectangular co-ordinates with respect to the more horizontal oblique lines gives the Ca·· of the serum, *e.g.* at 8 per cent. protein and 12 mg. Ca/100 c.c., the Ca·· is 5 mg./100 c.c. (McLEAN and HASTINGS, *Amer. J. Med. Sci.*, 1935, **189**, 601.)

clotting, and for the maintenance of the normal excitability and permeability of different types of cells.

The calcium of the blood is practically all in the plasma (or serum), and amounts to about 10 mg./100 c.c. Not all of it is diffusable or ionisable, but usually only about half of the total amount. Practically all that is dialysable is fully ionised. The fraction that is non-dialysable is attached to protein and in fact the calcium ion concentration of the plasma can be calculated from the formula of McLean and Hastings :—

$$\frac{[Ca^{\cdot\cdot}] \times [\text{Protein}]}{[\text{CaProteinate}]} = 10^{-2\cdot22} \text{ at } 25^\circ \text{ and } pH\ 7\cdot35.$$

A nomogram for finding the serum calcium from the total Ca and total protein is given in Fig. 516.

The ionic calcium also varies in the opposite direction to the pH of the plasma, but no strict relationship between them seems to have been yet discovered. The Ca of the cerebro-spinal fluid is all in the ionic state, and as a rule equals that of the ionic calcium of the blood plasma.

* LEITCH. *Nutr. Abstr. Rev.*, **6**, 553.

Soluble calcium salts, such as chloride, lactate or gluconate, given by mouth, cause an increase in the calcium of the plasma in an hour or two; a few hours later this returns to normal again. Amounts varying from 10 to 50 per cent. of the intake of calcium by the mouth are excreted in the urine, and from 50 to 90 per cent. are excreted in the fæces and probably represent unabsorbed calcium salts. But some state that when calcium salts are given by mouth there is no increase in the calcium of the fæces; on the other hand, it is claimed that in fasting the calcium content of the fæces is but little reduced.

Phosphorus is obtained partly in the form of phosphates of inorganic cations, but largely as organic phosphorus compounds. In the course of their metabolism in the body, these liberate phosphoric acid. Thus various phosphatides, nucleic acid derivatives, etc., all ultimately yield phosphoric acid. Part of the phosphorus present in cereals is in the form of phytin (inositol hexaphosphate), and this source of phosphorus is not utilisable in the body. Sources of available phosphorus are similar to those of calcium, but meat and fish, though poor in calcium, contain plenty of phosphorus.

Phosphorus, in the form of various derivatives of phosphoric acid, plays an important part in the processes of intermediary metabolism. Thus sugars and fats are metabolised through the intermediation of phosphorylated derivatives.

Phosphorus and calcium should be provided in a proper ratio, which in most diets is about 2 P to 1 Ca. Since both are principal constituents of bone and teeth, they must often be considered together.

7. BONE AND BONE FORMATION

Bone is a living tissue which consists of 25 per cent. water and 30 per cent. of an organic matrix impregnated with inorganic material. When a bone is soaked in dilute acid the inorganic material is dissolved away, and the remaining organic matrix is elastic and flexible. Calcination, by removing the organic matrix, leaves only the inorganic material as a friable residue of bone ash, forming some 45 per cent. of the weight of the original bone. The chief organic constituent is a collagenous protein called ossein. The inorganic material consists chiefly of calcium phosphate and carbonate, with some magnesium phosphate. Examination of the X-ray spectrum of bone shows it to be crystalline in structure, and to correspond to one of the minerals known as apatite or hydroxyapatite, *i.e.* either

$$3\ Ca_3(PO_4)_2.CaCO_3 \text{ or } Ca_3(PO_4)_2.Ca(OH)_2.$$

It is not proposed to describe here the histological features of bone formation. That the cartilaginous basis and the growing bone are formed by the osteogenic cells of the periosteum and endosteum has been shown by the direct method of tissue culture. Cartilage cells, or bone cells, when once formed, however, multiply as such, though the osteogenic layer of the periosteum can produce either cartilage cells or bone cells.

The foundation of cartilage on which a long bone is subsequently laid serves to determine roughly what the shape of the finished bone will be. It is, in fact, possible to cultivate *in vitro* a portion of undifferentiated mesenchyme from the thigh region, and to witness the emergence of a roughly formed femur in tissue quite removed from its usual surroundings. The final details of the architecture of the bones, and of the joints is, however, the result of the operation of various extrinsic factors.

In the development of the long bones, the primary spongy bone which at first replaces the scaffolding of cartilage is itself only provisional, and is

reabsorbed by the activity of "osteoclasts," the while new bone is being laid down to replace it. This simultaneous absorption and formation of bone is not confined to fœtal life or even to childhood, but continues, though at a slower tempo, throughout life, so that bones are far from being the static and finished structures they are commonly believed to be.

As a bone grows it increases in thickness by absorption from within the marrow cavity while sub-periosteal bone is being laid down on the outside. Hence, a layer of bone which at an early stage lies just beneath the periosteum comes at later stages to appear to sink into the substance of the bone, until it finally reaches the layer adjoining the medullary cavity, after which it disappears. This can be shown by feeding a growing animal for a time on madder. This colours the new bone red, and on cross section of the shaft of a long bone from the animal, the new bone deposited during the period

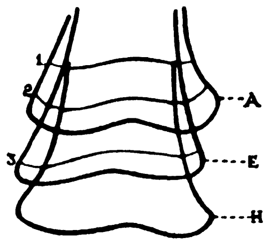

FIG 517 Superimposed radiographs of bones of a child taken at intervals over a period of 106 weeks (Radiographs by H A Harris) On right, outlines of three pictures of lower end of the femur Between A and E, 63 weeks elapsed, and between E and H, 43 weeks The lines of growth retardation corresponding to periods of illness are at 1, 2 and 3. On left, A and H with 106 weeks' interval, are of tibia of same child. Note how much bone has been re-absorbed to form the final shape of H in both cases (BRASH, *Edin Med Journ*, 1934, **41**, 305)

of madder feeding stands out clearly as a coloured ring. If the animal is killed at or shortly after the period of madder feeding, the coloured ring is at the surface of the shaft, but as the interval between feeding and killing is lengthened, so the position of the coloured ring is found to lie further and further away from the surface. This method of growth explains why it is possible to put a fœtal femur inside the medullary cavity of an adult femur, although we know that one has developed by a process of continuous growth from the other.

Long bones grow in length by progressive growth and ossification of the cartilaginous epiphyseal plate. During certain illnesses the rate of growth is greatly slowed, and the lamina of bone then laid down is more dense than normal, and remains so, as a permanent record visible in an X-ray of the bone. As this lengthening occurs it is accompanied by a good deal of re-moulding of the outer surface of the epiphysis by sub-periosteal re-absorption (Fig. 517).

Since the bones and teeth stand out from other tissues by reason of their high content of calcium salts, much attention has been directed to finding how this calcification has been brought about. It seems probable that the essential feature of the process consists in the construction, first of an organic matrix by the osteogenic cells, and then the calcification of this matrix by

the formation in it of a precipitate of calcium phosphate. The precipitated calcium phosphate then appears to adsorb ions of $Ca^{\cdot\cdot}$, OH', and CO_3' to form the apatite-like bone salt.*

Processes of calcification and of decalcification are apparently always going on in bone—sometimes, as in growing bone, calcification greatly exceeds decalcification, and sometimes, as when there is present an excess of the hormone of the parathyroid, decalcification is in excess of calcification. The two processes are probably independent, and occur locally as a result of cell activity, and in the normal adult a state of equilibrium between the two processes occurs. That continuous replacement of the inorganic material of bone goes on is shown by the fact that when salts of radio-active calcium or phosphates containing radio-active P are administered, the radio-active element can be detected in all the bones in a few hours or days.

The deposition of calcium phosphate in the formation of bone is probably bound up with the fact that the formation of a precipitate of an ionisable substance from a solution depends on the concentration of the constituent ions reaching a value at which their product exceeds the 'solubility product' of the substance concerned. In this case, when the solution is in equilibrium with a small amount of solid calcium phosphate, *i e*, when the solution is saturated, then $[Ca^{\cdot\cdot}] \times [PO_4''']=K$. Protein, by forming unionised Ca proteinate, increases the amount of Ca in solution and, as we have seen (p. 848), the concentration of Ca ions in plasma can be calculated if the total Ca and total protein are known. If the concentration of either the $Ca^{\cdot\cdot}$ or the PO_4''' in the saturated solution is increased, then calcium phosphate is precipitated until the product $[Ca^{\cdot\cdot}] \times [PO_4''']$ falls to the solubility product value. Precipitation of calcium phosphate from a saturated solution can thus be brought about by adding a soluble phosphate (in just the same way as we can throw down sodium chloride from its saturated solution by the addition of HCl), or alternatively by the addition of excess Ca ions in the form of a soluble calcium salt.

Now the product of the $Ca^{\cdot\cdot}$ and PO_4''' ions in normal blood plasma is about equal to the solubility product of Ca phosphate, and therefore an increase in, say, PO_4''' ions will throw down calcium phosphate; moreover, the calcium phosphate thrown down will not be redissolved unless the ionic product falls far below the solubility product.

It was shown by Robison that in the process of calcification an important part is played by an enzyme called *bone phosphatase*, which is produced by the osteoblasts. This enzyme can decompose phosphoric acid esters (such as glycerophosphate or hexose monophosphate), which are unionised, with liberation of PO_4''' ions, and so lead to such a local increase in the ionic product as to exceed the solubility product of calcium phosphate, which is therefore deposited from the blood plasma. The enzyme is present in ossifying (but not in non-ossifying) cartilage: similar phosphatases also occur in other tissues, especially in the small intestine and kidney; we cannot here discuss the interesting question as to why these last two tissues do not ossify, except to state that the bone matrix evidently has something to do with the deposition which occurs in bone. Bone salts are deposited on a piece of boiled ossifying cartilage placed in the abdominal cavity.

Phosphatase is not originally present in the cells of the mesenchyme from which the bone is to be formed, and the appearance and increase of phosphatase in a developing bone can be followed by making estimations of phosphatase on portions of a bone growing *in vitro* (Fig. 518).

* LOGAN and TAYLOR. *J. Biol. Chem.*, 1937, **119**, 293.

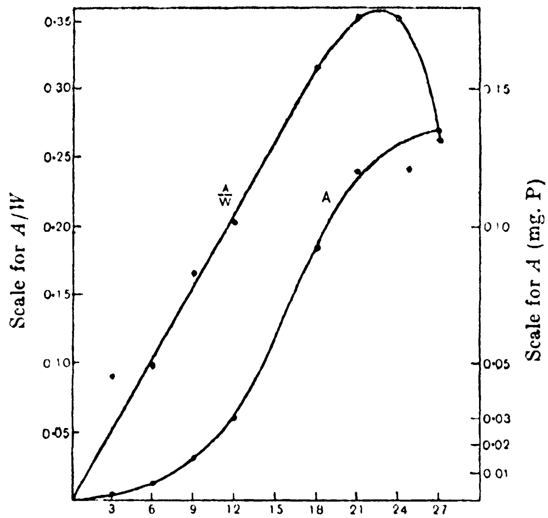

FIG. 518. Production of phosphatase during development of femora of fowl embryos excised at sixth day.
A = Phosphatase per femur (mg. P hydrolysed from glycerophosphate).
A/W = Phosphatase per mg. dry weight of femur (after FELL and ROBISON. *Bioch. J.*, 1929, **23**, 767).

It is perhaps significant that magnesium, which is also an invariable constituent of bone, greatly accelerates the action of phosphatase.

JOINTS

A joint is a tissue space lined by modified connective tissue cells. The articular surfaces are covered by hyaline cartilage and are kept in contact mainly by the supporting structures of the joint, such as the ligaments. The joint capsule is entirely of mesenchyme origin, and the inner layer of the synovial membrane is provided with blood vessels, lymphatics and nerves, and lined by large modified connective tissue cells which simulate an epithelium. The intermittent pressure to which a joint is subjected is important in keeping the articular surfaces in normal condition, and no doubt also in facilitating exchanges between the blood and the synovial fluid. The synovial membrane, unlike the articular cartilage, has high powers of regeneration if damaged, and is difficult to stretch.

THE SYNOVIAL FLUID is normally small in amount, *e.g.*, about 0·5 c.c. in a normal human knee-joint. It is a clear, pale yellow fluid, viscous and slippery to the feel, owing to the presence of mucin, and is subjected when *in situ* to slight if any positive pressure when the joint is at rest. It contains a number of cells, in man about 60 c.mm., though the number and type of cells varies considerably, no doubt as a result of the occurrence of slight degrees of trauma to the joint. Of the cells present in normal fluid about half are monocytes and a quarter lymphocytes ; the rest are polymorphs, and other cells similar to those of blood.

In composition, the fluid is distinguished by the presence of mucin, about 0·14 per cent., and it contains about 2 per cent. of other proteins. Inorganic ions are nearly the same as in the plasma, such difference as exists being what would be expected from the Donnan equilibrium if we regard the synovial fluid as being in equilibrium with the blood. The high Ca content is at variance with this, however. Other constituents, such as urea, uric acid, etc., are at the same concentration as in the plasma. The relatively low protein content, as compared with the plasma content of 8 to 9 per cent., together with the fact that the albumin/globulin ration is 3·9 as compared with 1·1 in the serum, means that, as in other body fluids, the passage of proteins from the blood stream is relatively difficult. It contains no fibrinogen and so does not clot.

There have been many suggestions as to how the synovial fluid is formed, but little experimental work. The most likely view is that the fluid is the liquid matrix of connective tissue, that the mucin originates therein, and that the fluid in this tissue space is in diffusion equilibrium with the blood. It has been abundantly demonstrated that substances of small molecular size pass readily from blood to synovial fluid, or *vice versâ*, and it is easy to show that acid dyes of high diffusibility pass freely from the blood into the joint.* Particulate matter, such as carbon particles, introduced into a joint, are slowly phagocytosed and removed by the lymphatics.

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* ENGEL. *Q. J. Physiol*, 1940, **30**, 231.

CHAPTER XLII

THE VITAMINS

If we feed an animal on a mixed diet containing proteins, fats, carbohydrates and salts in the proper proportions, but in a state of purity, *e.g.* a mixture of caseinogen, starch, sugar, salts and lard, we find that the animal becomes ill, ceases to grow if a young animal, and finally dies. This had already been pointed out in 1880 by Lunin, but the matter was taken up again by Hopkins,* who worked on young growing animals. He found that in the case of rats it was sufficient to add 3 c.c. of milk daily to the artificially compounded diet for growth to take place normally (Fig. 519). Three cubic centimetres of milk contain only 0·08 gramme total solids, of which the greater part consists of substances already present in the artificial diet; yet this small amount of fresh milk enabled the rats to add 0·5 gramme a day to their weight. These observations were the beginning of a lengthy series of experiments by many different observers. It has been found that practically all fresh foods contain extremely small traces of essential substances to which the name 'vitamins' or accessory food factors has been given. They are essential for the utilisation of the food, for the maintenance of health and for growth. Fifteen vitamins have been clearly distinguished according to their different properties and mode of action. In experiments on the vitamins, animals are fed for several weeks on a diet deficient only in the vitamin concerned, and are compared with controls fed with the diet, but containing the vitamin.

The vitamins are distinguished by letters. Some are soluble in fats and occur naturally together in association with them, while others are water-soluble.

TABLE OF VITAMINS

Fat Soluble Vitamins

A $\begin{cases} A_1 & \text{Also termed Axerophthol, the anti-xerophthalmic or anti-infective factor.} \\ A_2 & \text{Closely related to } A_1\text{, but of small importance.} \end{cases}$

D_2 (Calciferol) an artificial derivative of ergosterol.

D_3 Natural vitamin D.

E (Tocopherol). Anti-sterility factor.

K Anti-hæmorrhagic vitamin concerned with blood-clotting.

Water Soluble Vitamins

B_1 (Aneurin). Anti-neuritic. Beri-beri preventive factor.

B_2 (Riboflavin). Concerned with oxidising mechanisms.

$\left.\begin{matrix} B_3 \\ B_4 \\ B_5 \end{matrix}\right\}$ Growth factors detected in certain animal experiments; relation to human nutrition not yet defined.

B_6 Rat anti-dermatitis factor.

P.P Factor. (Nicotinic acid or Nicotinamide.) Anti-pellagra factor.

C (Ascorbic acid). Anti-scorbutic factor.

P Citrin or Hesperidin. A factor associated with ascorbic acid and believed to supplement its action.

* HOPKINS. *J. Physiol.*, 1912, **44**, 425.

FAT SOLUBLE VITAMINS

Vitamin-A_1 is essential for growth and its absence leads to failure of growth, excessive keratinisation of the skin, including an affection of the cornea called xerophthalmia, a general lowering of resistance to infection and ultimately to death.

Prolonged deficiency of this vitamin has also been shown to cause extensive degeneration in the central nervous system, especially in the afferent nerves and tracts.* Another effect of Vitamin-A deficiency is night-blindness, *i.e.* the inability to see in dim light. This is a consequence of reduction of visual purple in the retina owing to structural changes in the rods,† and also perhaps to a chemical relationship between visual purple and Vitamin-A. The occurrence of night-blindness is a convenient symptom of A_1-deficiency.

The influence of this vitamin on growth is illustrated in Fig. 519.

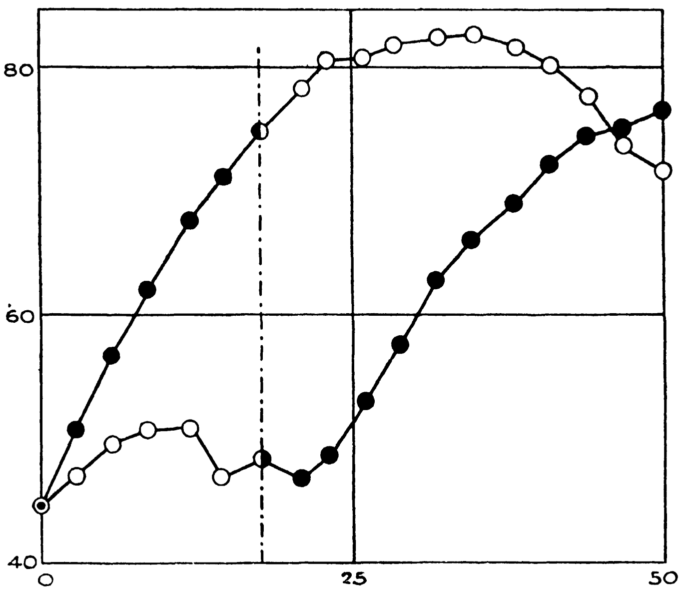


FIG. 519. Two Curves showing Growth of Rats with and without "Vitamin-A." Lower curve (up to 18th day), eight male rats on pure dietary, upper curve, eight similar rats taking 3 c c of milk each a day. On the 18th day, marked by vertical dotted line, the milk was transferred from one set to the other. Average weight in grammes vertical; time in days horizontal. (F. G. HOPKINS.)

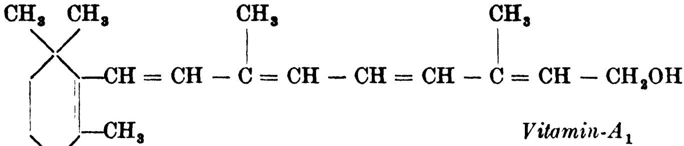
Vitamin-A is originally formed in the growing green plant, and it is doubtful whether any higher animals have the power of forming it *de novo* in their bodies. The cow, for instance, obtains its A-vitamin from grass: after ingestion it is absorbed into the fat of the body and of the milk, so that it is taken by man largely in the form of butter or milk. The organs richest in Vitamin-A are liver and kidneys. It is contained in large quantities in cod liver oil, and in even larger amounts in the oils from mammalian liver. Its origin in the sea is especially in the green diatoms which serve as food for the small plankton, organisms which are the basis of the food supply of the

* E. MELLANBY. *Brain*, 1935, **58**, 141.

† TANSLEY. *J. Physiol.*, 1931, **71**, 442; *Proc. R. S.*, 1933, **114B**, 79.

larger fish. It is not destroyed by heat, though easily oxidised. It is absent from most specimens of lard and from many vegetable oils, such as olive oil. If, however, pigs are fed on green food, pig fat contains the vitamin. It is much more abundant in the milk of cows in summer, out at grass, than when these animals are kept in stables and fed on oil-cake, &c. It can be stored in the body to a certain extent, so that the effects of its absence from the food are not perceptible in adults till after a few weeks. It can be concentrated from the other extracts of materials that contain it; the fats can be removed by saponification in the absence of oxygen.* The residue can be freed from the cholesterol and still contains the vitamin, which persists in the fraction consisting of unsaturated alcohols and hydrocarbons, but in very minute quantities.

Vitamin-A_1 is a viscid colourless oil of the elementary composition $C_{20}H_{30}O$. Its structure † is:



Vitamin-A_1

It is an unsaturated alcohol, insoluble in water but soluble in all organic solvents, and related to the unsaturated hydrocarbon carotene, $C_{40}H_{56}$. This is a crystalline pigment which gives a yellowish colour to certain natural fats and oils. Both carotene and Vitamin-A give a blue colour when treated with antimony trichloride in chloroform, and may be estimated colorimetrically by this means. Another method is by the determination of the coefficient of absorption in the ultra-violet (328 mμ). It would seem that, although animals cannot synthesise Vitamin-A, they can partially convert carotene into it. The amount of the purest Vitamin-A which is required to keep a rat from developing symptoms of Vitamin-A lack is only 0·1 to 0·5 μg. daily, whereas about 3 to 5 μg. of carotene is needed (1 μg = 0·001 mg.). Carotene is thus to be regarded as the pro-vitamin of A. Probably the change into Vitamin-A occurs in the liver, in which the greater part of it is then stored. The League of Nations has selected pure β-carotene as a provisional standard for Vitamin-A. The unit is 0·6 μg, which is equivalent in activity to 0·33 μg of pure Vitamin-A.

The requirement for human beings is about 75 units per kg. body-weight per day, or, say, for an adult, about 5,000 units daily. Probably many diets only supply about 1,000.

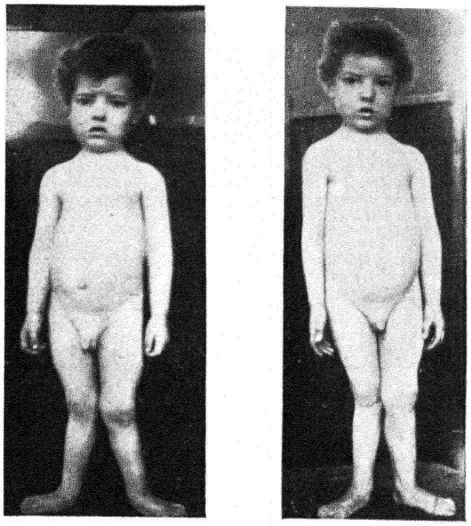
Milk contains about 3–5 units per c.c.; butter about 20–50, egg yolk about 80, spinach 100, carrots 20 units per g., and other vegetables usually much less.

Vitamins-D. It was shown by E. Mellanby,‡ that the absence of growth in vitamin lack is associated with abnormal formation of bones, which gives rise to the changes associated with the disease of *rickets* (Figs. 520, 521). An important factor in the causation of rickets is the absence of Vitamin-D, which is therefore often called the anti-rachitic vitamin. Vitamin-D also produces growth effects to a minor extent, but its chief effect seems to be in controlling the balance of calcium and phosphorus. In normal plasma

* McCollum and Davis, *J. Biol. Chem.*, 1914, **19**, 245; Drummond and Coward, *Lancet*, 1921 (ii) 698; Drummond, Channon and Coward, *Biochem. J.*, 1925, **19**, 1047.

† Heilbron, Heslop, Morton, Webster, Lea and Drummond. *Bioch. J.*, 1932, **26**, 1178.

‡ E Mellanby. *J. Physiol.*, 1918, **52**, xi, liii; *Lancet*, 1919 (i) 407.



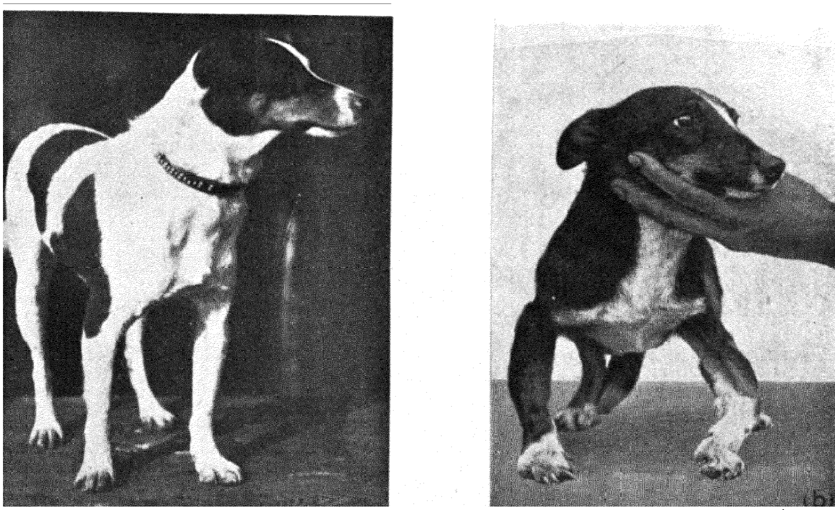
(*a*) (*b*)

FIG. 520. Improvement in Genu Valgum by a Calcifying Diet

(*a*) Child aged five years, with leg deformity caused by rickets

(*b*) Improvement after six months' treatment on a highly calcifying diet

(E. MELLANBY. "Nutrition and Disease." Oliver and Boyd, 1934.)



(*a*) (*b*)

FIG. 521.

(*a*) Normal dog reared on a good diet rich in Vitamin-D during a period of confinement.

(*b*) A rickety dog brought up on a diet similar to that of (*a*) except that it lacked Vitamin-D.

(E. MELLANBY. "Nutrition and Disease." Oliver and Boyd, 1934.)

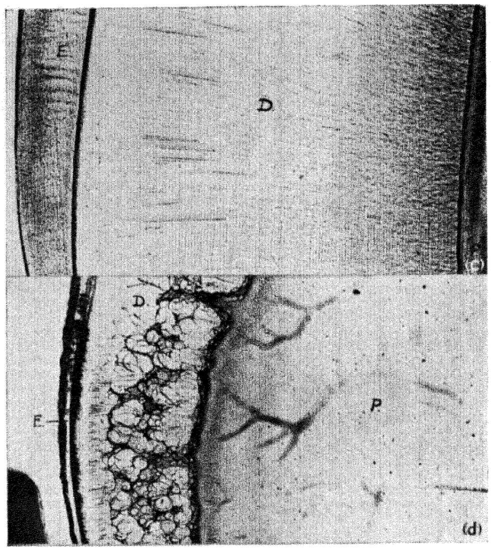


FIG. 522. Carnassials of Two Puppies.

Upper = Received cod-liver oil. Normal enamel and dentine.

Lower = Received no fat-soluble vitamin. Thin and badly calcified enamel and dentine, and many interglobular spaces. E = enamel. D = dentine. P = pulp cavity

(MAY MELLANBY. Taken from E MELLANBY, "Nutrition and Disease," Oliver and Boyd, 1934.)

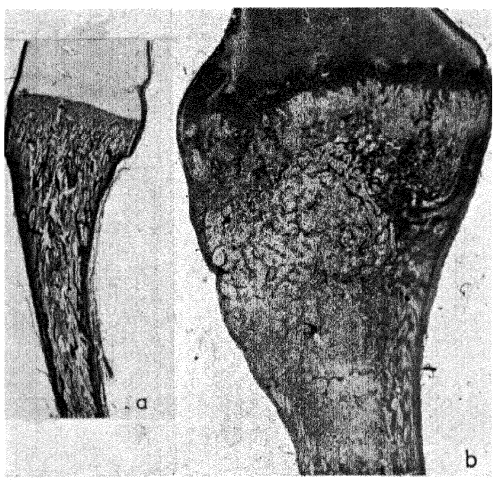


FIG. 523 Costo-chondral junctions of normal (*a*), and rickety (*b*) dogs.

The diets of the two dogs contained a large amount of oatmeal, which is rickets-provoking, but that of (*a*) contained cod-liver oil, while that of (*b*) contained olive oil

(E MELLANBY. "Nutrition and Disease," Oliver and Boyd, 1934.)

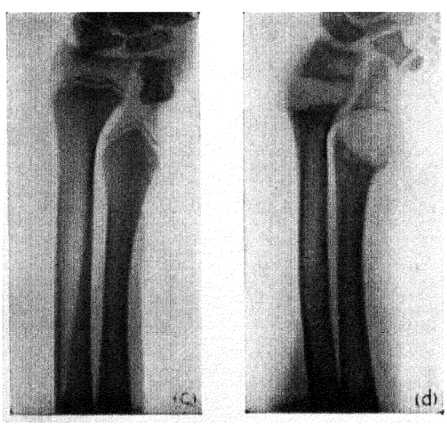


FIG. 524. (*c*) Radiograph of wrist of dog (*a*): normal (*d*) Radiograph of wrist of dog (*b*): rickets.

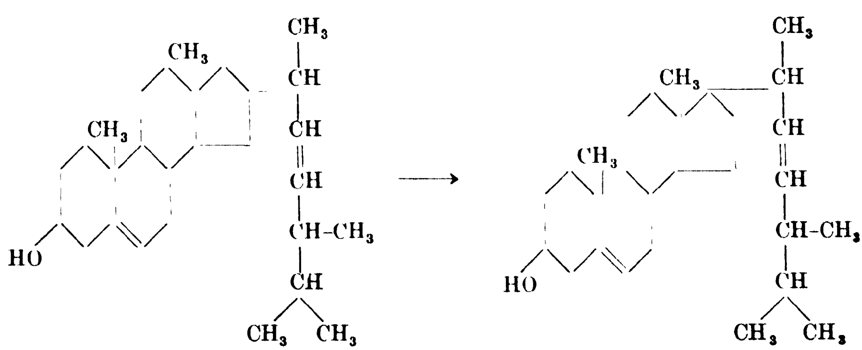
(E. MELLANBY. "Nutrition and Disease," Oliver and Boyd, 1934)

[*To face p.* 857.

the product of the total Ca and P (inorganic) in mg./100 c.c. is over 50; in rickets both fall, the P first, and the product is below 40. It has also been demonstrated by May Mellanby that Vitamin-D plays an important part in the formation of the teeth (Fig. 522). Once the dentine has been formed, however, administration of Vitamin-D cannot alter it either in structure or composition.*

The most marked change in rickets is a defect in the ossification normally occurring at the epiphyseal line (Figs. 523-524). Ossification in cartilage consists of the following stages: (1) multiplication of cartilage cells; (2) calcification of the cartilage; (3) absorption of the calcified cartilage; (4) deposition of true bone, around cells—osteoblasts—which have invaded the calcified cartilage from the marrow. In rickets there is an accelerated multiplication of cartilage cells and a retardation in the deposition of true bone, from a failure in the process of deposition of calcium salts. It is evident that in the causation of rickets, or for the growth of normal bone, many factors are involved. In the first place, the presence of Vitamin-D is necessary. This may, however, be replaced to a certain extent by exposure to the sun's rays. Then it is necessary that calcium salts shall be present in proper amount. Deficiency of calcium and phosphates in the diet may therefore hinder the formation of bone. Thus cereals have a rickets-promoting effect owing to the fact that the phosphorus is present in a non-assimilable form as phytin, and also because the phytic acid inhibits absorption of calcium.† It has been shown by Robison that, in the carriage of calcium salts to the bone, a part may be played by organic phosporic esters which form soluble salts with calcium, and which have been detected in blood. Under the action of phosphatase which is present in ossifying cartilage, and which is *also present in rachitic bone*, this calcium salt is hydrolysed, and calcium phosphate is precipitated in the matrix round the osteoblasts. The manner of action of Vitamin-D is unsettled, but it is probable that it primarily attacks the lipides of the tissues, with liberation of phosphoric acid,‡ the lack of which in the blood is a notable feature of rickets. It prevents loss of calcium by the bowel by causing its deposition as phosphate. As a net result, Vitamin-D acts by allowing an increased net absorption of calcium and phosphate, so raising their levels in the blood.

It is interesting that exposure of animals to sunlight, or to ultra-violet light, can replace to a certain extent the necessity for the presence of D-vitamin in the food. Investigation of the cause of the similarity between the effect of addition of Vitamin-D to the diet and exposure of the animal to ultra-violet light, led Steenbock to the remarkable discovery that Vitamin-D-activity is produced by the action of ultra-violet light on a precursor which is present in the tissues, including the skin. It was further found by Rosenheim and Webster § that this precursor, frequently found in association with cholesterol, is another sterol called *ergosterol*, $C_{27}H_{42}O$. Ergosterol has a



Ergosterol *Calciferol* (*Vitamin-D_2*).

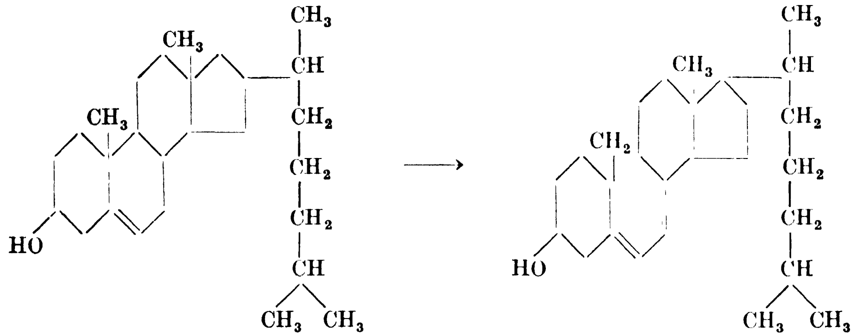
* FISH. *J. Physiol.*, 1935, **84**, 272.
† HARRISON and MELLANBY. *Bioch. J.*, 1939, **33**, 1660.
‡ MCGOWAN *et al.* *Bioch. J.*, 1931, **25**, 1295.
§ ROSENHEIM and WEBSTER. *Biochem. J.*, 1926, **20**, 537; 1927, **21**, 389.

characteristic absorption spectrum in the ultra-violet region. Irradiated ergosterol has many of the properties of Vitamin-D.

By distillation of irradiated ergosterol in high vacuum, a crystalline product of double its activity was obtained; this was called 'calciferol,'* or Vitamin-D_2, and is an isomer of ergosterol. In large doses it causes loss of weight, toxic symptoms and death; extensive deposits of calcium salts are found in the heart, great arteries and kidneys. The antirachitic activity of calciferol is 40 million units per milligramme and it is called Vitamin-D_2.

The League of Nations standard unit for Vitamin-D activity is 0·025 μg. of pure calciferol or of Vitamin-D_3.

It now appears that there are various natural Vitamins-D, the chief of which, D_3, is a very close isomer of cholesterol. Its actions are identical with those of D_2.

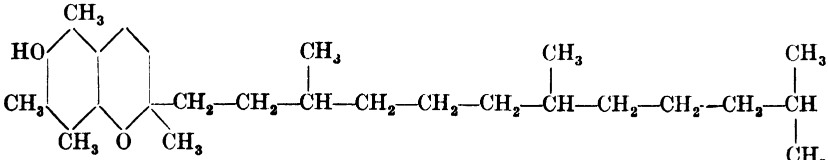


Cholesterol. *Natural Vitamin-D (Vitamin-D_3) or activated γ-dehydrocholesterol).*

The human daily requirements of Vitamin-D are not precisely known, but it is probable that growing children need more (*e.g.* 2,000-5,000 units) than adults (200–500 units). It is essential that adequate amounts of calcium (*e.g.* 1·5 g./day) and of phosphorus (2·0g./day) be also available. Poor dietaries lack these, as well as the Vitamin-D. Butter, cream and egg yolk are richest among ordinary foods; fish liver oils are especially rich.

Vitamin-E. Recent experiments indicate that, for reproduction to take place, another vitamin may be necessary. Animals deprived of this vitamin may thrive and be to all appearance perfectly well, but do not produce young. Conception takes place, but the embryo fails to attain full development, dies *in utero* and becomes absorbed. Vitamin-E is present in wheat-oil, butter and certain other fats. It is present in the unsaponifiable fraction, is not destroyed by light, heat, acids, alkalis, oxidation or hydrogenation, or distillation *in vacuo,* and has an absorption band of 294 mμ.

The human requirement is probably 3 mg. per day. It possibly acts through the pituitary. There are several related components which are alcohols of the formula $C_{29}H_{48}O_2$, called the tocopherols. The formula of α-tocopherol is:—



α-Tocopherol.

* **Angus** *et al.* *Proc. Roy. Soc.*, 1931, **108B** 340, (and simultaneously by four other groups of investigators).

Vitamin-K * is another fat soluble vitamin, lack of which, in animals, produces symptoms rather like scurvy and involving multiple hæmorrhages in skin and muscles, associated with greatly decreased clotting time of the blood. It is present in many vegetable substances, especially cabbage and spinach. Its action is believed to be associated with alteration in the prothrombin content of the plasma. Bile is essential for its absorption. Chemically it is a methyl-phytyl-naphthoquinone.†

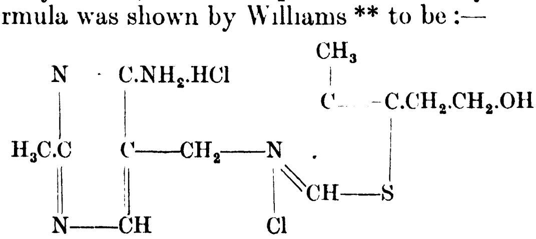
WATER SOLUBLE VITAMINS

The Vitamin-B Complex. In races whose staple diet consists of rice, prolonged feeding with polished rice, *i.e.* rice from which the husks have been removed, leads to the production of the disease known as beri-beri. This is distinguished by the occurrence of pains and weakness in the limbs, loss of sensation in the skin, œdema, and weakness of the heart. A somewhat similar disease may be produced by the same means in fowl or pigeons. The disease may be cured by adding the polishings, *i.e.* the part of the rice grain which has been removed, to the polished rice before eating. It is evident that some substance, which is essential to the normal nutrition of the body, is removed during polishing. Since it can be subdivided into a number of nearly related vitamins, it is now referred to as the 'Vitamin-B complex.' ‡ It is not stored to any extent in the body, so that animals rapidly show the effects of its withdrawal. Absence of B-vitamin leads in rats to wasting, failure of growth, atrophy of the testes and sterility. The wasting is due to lack of appetite and to digestive disturbances. Secretion of gastric juice is reduced.§

The individual vitamins in the complex are six in number; the two most clearly defined have been called B_1 and B_2; they can be differentiated by their physiological effects and by the action of heat and alkali, either or both of which destroy B_1. Though both are necessary for growth, Vitamin-B_1, or 'aneurin,' is believed to be related to beri-beri, while B_2 has important functions in oxidising mechanisms. Yeast is rich in both, wheat germ in B_1, and milk, meat and green vegetables in B_2.

Vitamin-B_1. Among the effects of Vitamin-B_1 is a quickening of the rate of the heart-beat, which is slow in its absence.|| Further, in lack of B_1 there is an accumulation of lactic and pyruvic acids in the blood and especially in the brain.¶ Convulsive symptoms in pigeons in extreme lack of B_1 are probably due to this; when Vitamin-B_1 is added to the brain of such pigeons *in vitro* the oxygen utilisation of the tissue is raised. It is therefore probable that this vitamin plays some part, perhaps that of a co-enzyme for pyruvate carboxylase (which co-enzyme is the pyrophosphate of aneurin), in the metabolism of carbohydrates.

Crystalline, Vitamin-B_1, was isolated by Windaus and by Otake; its formula was shown by Williams ** to be:—



Vitamin-B_1 (Hydrochloride of Aneurin).

* Dam *et al.* *Bioch. J.*, 1936, **30**, 1075; 1938, **32**, 485.
† Binkley *et al.* *J. Biol. Chem.*, 1939, **130**, 219.
‡ Nelson. *J. A. Med. Assoc*, 1938, **110**, 645.
§ Webster and Armour. *Proc. Soc. Exp. Biol Med.*, 1934, **31**, 463.
|| Birch and Harris. *Bioch. J.*, 1934, **28**, 602.
¶ Kinnersley and Peters, *Bioch. J.*, 1930, **24**, 711.
** Williams. *J. Amer. Chem. Soc*, 1936, **58**, 1063.

It has been synthesised.*

The standard for Vitamin-B_1 recommended for international adoption is a crystalline preparation of the pure substance. The international unit has been defined as 3 μg. of this. The requirement depends on the rate of metabolism. For average human individuals it is about 300 units (1 mg. aneurin) daily. Up to 25 per cent. of ingested Vitamin-B_1 is excreted in the urine. Symptoms of lack include fatigue, loss of appetite, dyspnœa on exertion, muscle cramp and skin hyperæsthesia.

The daily requirements for man are related to the calorific value of the diet, and can be calculated from Cowgill's formula :—

$$\text{Vitamin } B_1 \text{ intake (in International Units)} = \text{Calorific intake} \times \text{Wt. (Kg.)} \times 0{\cdot}0284.$$

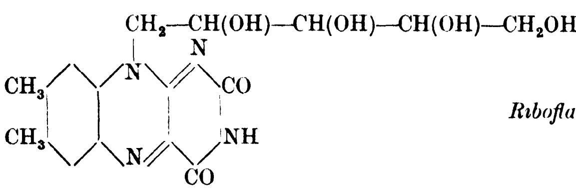
The minimum requirement of an adult taking a daily diet of about 3,500 calories would thus be about 550 International Units. Many modern diets supply only about 350. The beri-beri 'danger-line' appears to be about 200.

Children probably require about 400 units daily.

There is, however, considerable danger of Vitamin-B_1 deficiency in patients on restricted diets, or where individuals cut their own food intake down deliberately. The polyneuritis of chronic alcoholism is often traceable to shortage of Vitamin B_1 consequent on reduced food intake.

Vitamin-B_2 was formerly thought to be a complex, but has now been resolved into two substances, Vitamin B_2 and the P–P factor.

Vitamin-B_2. also known as lactoflavin (and sometimes also as Vitamin-G), is more correctly called *riboflavin.* Its proper name and structural formula is shown below :—

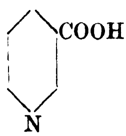


Riboflavin.

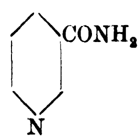
6.7. dimethyl-9-ribityl-isoalloxazin.

It is a yellow substance, present in milk whey, liver, kidney, heart, etc., which promotes growth, prevents cataract, keratitis and alopecia in rats, but does *not* prevent pellagra. It is an important constituent of the 'yellow respiration enzyme' of Warburg (*v.* p. 105), and hence plays an important part in oxidation processes. The daily requirement for man is 2–3 mg.

The P–P Factor is the vitamin which prevents pellagra, a disease prevalent in peoples feeding mainly on maize. The disease called 'black-tongue' in dogs is similar. The substance has been shown to be either nicotinic acid, or its amide, either of which will cure pellagra in man† or



Nicotinic Acid.



Nicotinamide.

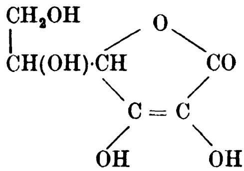
* Williams and Cline. *Ibid.*, p. 1504.

† Spies, Cooper and Blankenhorn. *J. A. M. A.*, 1938, **110**, 622.

black-tongue in dogs.* It is widely distributed in most foodstuffs, though maize contains but little.

It is worthy of note that, once more we have a vitamin which functions as a co-enzyme. In this instance, nicotinic acid is part of the co-zymase complex and therefore probably plays a part in carbohydrate metabolism (pp. 105, 157).

Vitamin-C. **This may also be called the antiscorbutic vitamin. Scurvy is a deficiency disorder which has been long known: it occurs when men are cut off for a long time from fresh food, especially vegetables. Its main** symptoms are weakness, anæmia, skin eruptions, small hæmorrhages under the skin, liability to infection and hæmorrhage from the gums and mucous membranes (p. 668). Captain Cook in 1776 † showed that it could be prevented or cured in sailors by the administration of malt or fresh vegetables. Oranges and lemons are specially rich in C-vitamin. We now know ‡ that Vitamin-C is identical with *l-ascorbic acid*,§ a hexuronic acid of the structural formula :—



l-ascorbic acid.

which was isolated from oranges, and also from the suprarenal cortex by Szent-Györgyi.|| In an acid medium this vitamin will stand exposure to boiling point for some time, but it is destroyed by oxidation almost at once on heating in an alkaline medium. The practice of adding alkali to preserve the colour of vegetables when they are cooked is therefore bad. This vitamin is present in small quantities in fresh meat and eggs. It is absent from most grains, but is formed when these grains germinate. When fresh vegetable food cannot be obtained, scurvy may be prevented by feeding with beans which have been moistened, and allowed to germinate in a warm place for twenty-four to forty-eight hours, or by giving the pure ascorbic acid.

Vitamin-C probably plays some part in the oxidation-reduction processes of the cell, acting as a hydrogen transporter. In some way also it is essential for the action of immunological complements of blood serum.¶ It has recently been shown that lack of Vitamin-C may be a factor in the promotion of dental caries; at all events it leads in guinea-pigs to arrest of formation of enamel and cementum. Corresponding to the absence of calcium salts from the matrix of bone and dentine formed during rickets, it has been shown ** that there is a breakdown in the formation of the organic collagenous part of the matrix during an attack of scurvy. This is well seen in the persistently growing teeth of the guinea-pig if the animal is deprived of Vitamin-C for fifteen to twenty days. The normal dentine produced before the attack appears in strong contrast to the irregular linear deposit of

* GOLDBERGER, JOSEPH and WHEELER. *Publ. H. Rep*, 1928, **43**, 172.

† COOK, J. *Phil. Trans. Roy. Soc.*, 1776, **66**, 402.

‡ SVIRBELY and SZENT-GYÖRGYI, *Nature*, 1932, **129**, 576; *Bioch. J.*, 1932, **26**, 865; WAUGH and KING, *Journ. Biol. Chem.*, 1932, **97**, 325; HARRIS and RAY, *Bioch. J.*, 1933, **26**, 2067.

§ The American Council of Pharmacy and Chemistry have adopted the name "cevitamic acid" for the same substance. (*v.* "Glandular Physiology and Therapy," 1935, p. 301. A.M.A.)

|| SZENT-GYÖRGYI. *Bioch. Journ.*, 1928, **22**, 1387.

¶ ECKER *et el.* *J. Immunol.*, 1938, **34**, 19.

** FISH and HARRIS. *Phil. Trans.*, 1933, **223B**, 489.

calcium salts (Fig. 525 (A)) held together by distorted traces of organic matter which is thrown down during the attack. Though lime salts are freely deposited, there is no attempt at this time to elaborate the usual tubular collagenous matrix for their reception. If the animal is restored to a normal diet for ten days before being killed, fresh odontoblasts are seen to have developed and to have provided the tooth with a new normal growing end. At the same time a belated mass of calcified collagenous matrix is deposited amongst the old degenerated odontoblasts. These, however, are now too broken up to leave their normal tubular pattern in it, so that this calcific scar consists of an amorphous mass of dentine matrix containing the *débris* of the disintegrated cells (Fig. 525 (B)). It represents the dentine which should have been laid down during the attack of scurvy, but which did not form then because the collagenous matrix was not provided.

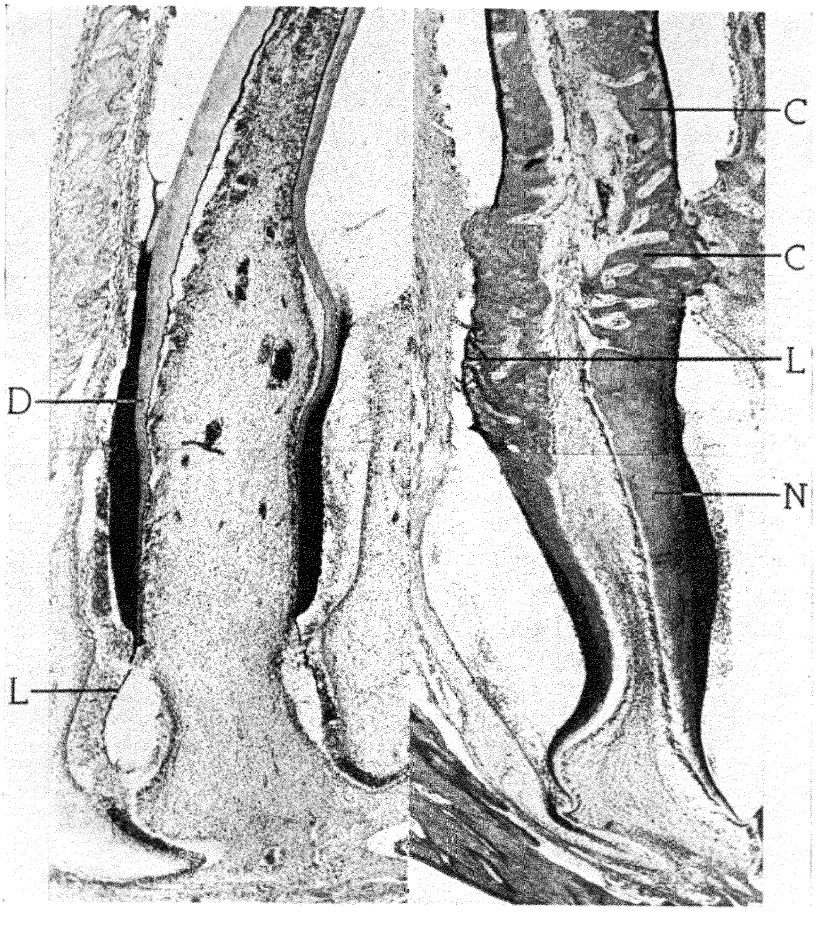
For experimental work on Vitamin-C, it is usual to employ guinea-pigs. The international unit is 0·05 mg. pure *l*-ascorbic acid, of which lemon juice contains about 10 units per cubic centimetre. A young guinea-pig requires about 0·7 mg. per day to prevent scurvy.* The fœtus receives its store of ascorbic acid from the mother. Children and adults require about 1,000 units (50 mg.) of ascorbic acid daily. It is best supplied by fruits, *e.g.* lemons, oranges and red currants, or vegetables. The amount of Vitamin-C in materials can be determined chemically by titration with "redox" dye

VITAMIN CONTENT OF FOODS

Food.	A Int Units per 100 gm or 100 c c	B_1 mg per 100 gm or 100 c c	C mg per 100 gm or 100 c c	D Int Units per 100 gm or 100 c c
Butter . . .	2,000–5,000	0·03–0·3	—	10–100
Cheese (Cheddar) .	5,500	0·1	—	—
Egg Yolk . .	8,800	0·3	0–1·3	100–250
Milk (cow's) . .	300–750	0·07	0·6–2·8	1·7–3·8
Bacon . . .	30	0·3	Trace	—
Beef, lean . .	120	0·1–0·3	1·8	—
Liver . . .	6,000–160,000	0·5	25–75	up to 50
Fish . . .	up to 1,500	0·1–0·2	1·7	100–800
Fish Roe . .	5,000	0·25	4·0–14·0	250–990
Apples . . .	70	0·125	0·1–20	—
Lemon Juice . .	—	0·05	25–70	—
Nuts . . .	100	0·24–0·9	15–30	—
Tomatoes . .	4,280	0·12	12–40	—
Cabbage, etc. . .	900	0·07–0·25	20–124	—
Carrots . . .	2,000	0·18	1·8–31	—
Peas . . .	800–1,300	0·12–0·3	4·8–40	—
Potatoes . .	35–70	0·1 -0·2	10–36	—
Spinach. . .	12,230	0·06–0·21	6–124	—
Bread, white . .	—	0·036–0·1	—	—
Bread, wholemeal .	—	0·16–0·25	—	—
Oatmeal . .	0–20	1·0	—	—
Rice, whole . .	50	0·06–0·27	—	—
Rice, polished .	—	—	—	—
Yeast . . .	—	1–12	—	—

* LUND, SPUR and FRIDERICIA. *Bioch. Journ.*, 1934, **28**, 1825.

PLATE III.



A B

FIG. 525.

A. Growing end of tooth of guinea-pig, deprived of Vitamin-C for fifteen days and then killed Photomicrograph × 115

D Normal dentine formed before the deprivation of Vitamin-C.
L. Linear deposit of calcium salts **deposited** in place of dentine during **attack.**

B. Growing end of tooth of guinea-pig deprived of Vitamin-C for twenty **days,** then transferred to full diet for ten days and killed. **Photomicrograph × 115.**

N. Normal dentine formed since the attack.
C. Calcific scar formed since the attack.
L. Linear **deposit** of lime **salts** deposited in place **of dentine during the attack of** scurvy.

(Courtesy of DR. E. W. FISH.)

(2 : 6 dichlorophenolindophenol).* By this means it can be shown that Vitamin-C is normally excreted in the urine, the daily amount being 1 to 2 mg. in children and up to 30 mg. in adults. Scorbutic children excrete very much less than normal amounts. If a large dose (70–100 mg.) of Vitamin-C be given to a normally nourished individual it is rapidly excreted, whereas when given to scorbutics the output is scarcely altered, showing that the substance is not excreted until a certain level of saturation has been reached.† The vitamin may also be estimated by a ferricyanide reduction method,‡ and spectrometrically.

Vitamin-P is found together with ascorbic acid, and its lack is largely responsible for the capillary hæmorrhages which occur in scurvy. It is also called citrin.

"*Citrin*," found in the rind and juice of citrous fruits, is a mixture of two flavone pigments, viz., a rhamnose-glucose glycoside of hesperidin, and a glycoside of eriodictyol. The human requirement is stated to be 50 mg. daily.

VITAMIN CONTENT OF FOODSTUFFS. The vitamin content of some common foodstuffs is shown in the Table on p 862.

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* HARRIS and RAY. *Bioch. J.*, 1933, **27**, 303.

† HARRIS and RAY *Lancet*, 1935, i. 71.

‡ TAUBER and KLEINER. *J. Biol Chem*, 1935, **108**, 563

CHAPTER XLIII

THE NORMAL DIET OF MAN

EXAMINATION of the actual requirements of the normal human individual involves a consideration not only of the total energy requirements of man according to age, size, sex and occupation, but also of the proper distribution of the foodstuffs in the diet.

We have seen that the energy requirements are a function of the body surface. The average height and weight of English adults is 171 cm. and 70·3 kilos., corresponding to a surface of 1·8 square metres (Fig. 513, p. 827). At 40 Calories per square metre per hour the average man will thus have an hourly basal metabolism of 71 Calories. When he is taking food this amount must be increased by about 10 per cent., and if he is up and about a further increase in metabolism will be evoked by the muscular movements, exposure to cold, &c. If we divide the twenty-four hours into three portions—eight hours' sleep, sixteen hours awake—(eight hours' work)—we must allow the basal metabolism for the eight hours' sleep, basal metabolism increased by about 30 per cent. for the eight hours during which the man is up and about and taking meals, and the basal metabolism plus an additional amount for work during the eight working hours. It is by no means easy to decide what figure should be accepted as representing the average work of an adult man. It is usual to take this as involving an energy output of 1000 Calories. Of these Calories 20 per cent., or even more, may under favourable conditions be transmuted into mechanical work, so that 1000 Calories should be sufficient to provide for the performance of about 100,000 kilogrammetres of external work. With these assumptions we arrive at the following energy requirements for an average adult working man :

8 hours' sleep at 71 (Basal Metabolism) . . .	568	Calories.
8 hours awake at 92 (Basal Metabolism plus 30 per cent.)	736	„
8 hours' work (Basal Metabolism plus 1000 Calories) .	1568	„
Total	2872	„

Some additional expenditure of energy will be involved in travelling, so that we are justified in accepting the figure usually given for the daily energy output of an average working man as 3000 Calories. For purposes of computation, about 400 Calories are usually added to allow for loss in preparation and in digestion, so that for the average man about 3400 Calories must be purchased.

A man in sedentary employment—*e.g.* typewriting, clerking, &c.—may not expend more than 200 to 400 Calories in the performance of external work ; mental exertion causes no appreciable rise in the metabolic exchanges. On the other hand, a heavy worker, such as a navvy, may easily expend 2000 or more Calories a day in the performance of external work.

The energy requirements of women will show corresponding variation according to occupation, but will be in general less in consequence of their smaller size and surface. Du Bois has shown that women have a lower basal metabolism per square metre of surface, viz. 37 Calories per hour as against 40 in the case of men. The average height and weight of Englishwomen are 159·3 cm. and 55·7 kilos., corresponding to a surface of 1·511 square metres and to an hourly output of 56 Calories. In estimating the total energy requirements of an average woman, we may reckon her work as two-thirds

that of a man, *i.e.* as 660 Calories per day. We then arrive at the following Table for the energy requirements of the average woman :

8 hours' sleep at 56	448 Calories.
8 hours awake at 73 (Basal Metabolism plus 30 per cent.)	584 ,,
8 hours' work (Basal Metabolism plus 660 Cals.) . .	1108 ,,
Total	2140 ,,

We shall not be far wrong, then, in accepting the usual estimate of a woman's net requirements as four-fifths of those of a man, viz. 2400 Calories, or the gross value as purchased as 2800 Calories.

In children a computation of the energy requirements is rendered more difficult by two factors. In the first place the metabolism is more energetic than in the adult, even if we take into account the relative surfaces in the two cases. Thus the basal metabolism per square metre in boys of varying ages was found by Du Bois to be as follows :

Mean age.	Basal metabolism, Cals. per square metre per hour.
6·5	57·5
12·6	50·4
13·7	49·4
16·5	43
19·25	40·7

In addition to this increased basal metabolism, if the processes of growth are to occur normally, there must be an excess of matter taken in over that excreted, since the growing child is always putting on weight. Between the ages of eleven and sixteen both sexes put on weight at about 4 kilos. a year, which is equivalent to adding to the body a store of about 800 Calories per month. The second difficulty is due to the fact that children are in a constant state of muscular activity, which is of great value in procuring healthy nervous and muscular development. The degree of this activity seems related to the amount of food taken—the increased energy of a child directly after a meal is a familiar phenomenon. If food is withheld or diminished this activity declines, but in this way energy is saved and the whole of the lessened food may go to maintain the temperature of the body and the normal processes of growth.

We may thus obtain a development of the child not differing appreciably from the normal on widely varying amounts of food, and it becomes difficult to say what is the average or the optimum amount that a child should have.

The B.M.A. Report expresses the requirements of the different sexes and ages in terms of the ' average man ' taken as 1, and it would appear that the amounts do not err on the insufficient side.

Man-values at Ages (Cathcart and Murray) (B.M.A. Report, 1933)

Ages	Man-value.	Calories.
Adult male	1 00	3400
,, female	0 83	2840
Child 1 and under 2 years . .	0·30	1020
,, 2 ,, 3 ,, . .	0·40	1360
,, 3 ,, 6 ,, . .	0 50	1700
,, 6 ,, 8 ,, . .	0 60	2040
,, 8 ,, 10 ,, . .	0 70	2380
,, 10 ,, 12 ,, . .	0 80	2720
,, 12 ,, 14 ,, . .	0·90	3000
Old person (65 +)	0 75	2550

The figures in the last column represent the Calories as purchased for the average individual, and are therefore equivalent to the food which must be digested and absorbed plus any loss. No food undergoes complete digestion,

the average loss being about 10 per cent. with an ordinary mixed diet, as is shown in the following Table :

Percentage of Foodstuffs Absorbed.

	Protein.	Fat.	Carbohydrate.	Ash.	Total Energy
Average of 5 experiments .	92·6	94	97·7	77·4	90·5

A further allowance must be made for unavoidable waste in cooking, &c.

Three thousand net Calories (*i.e. energy* requirements) require therefore the purchase of food with a Calorie value of 3400.

If the food consists mainly of vegetable products it may be necessary to increase still further the allowance for loss in digestion, not more than 80 per cent. of the protein being absorbed as a rule.

These values are for average well-nourished individuals. It has already been pointed out that in a state of semi-starvation, accompanied by loss of weight, the basal requirements may be reduced considerably. As a result of a number of experiments Chittenden * came to the conclusion that there was a definite advantage to be gained by reducing not only the protein intake of the body but also the total food. But the experience of Germany during the last three years of the great war showed that a forced limitation of food to about two-thirds of what we have put down as normal, resulted finally in a decrease of efficiency of mind and body and in a marked diminution in the resistance to infection, especially tuberculosis.

Distribution of Foodstuffs in a Normal Diet. There is general agreement that the average diet of an adult man should be somewhat as follows :

DAILY REQUIREMENTS OF AN ADULT MALE OF CALORIES, PROTEIN, FAT, AND CARBOHYDRATES (B.M.A. Report, 1933)

Total Calories required : 3400 in food as purchased

Derived from—	Quantity	Percentage of total Calories	Number of Calories
First-class protein .	50	6	205
Second-class protein .	50	6	205
Fat	100	27	930
Carbohydrate . .	500	61	2050
Totals . . .	700	100	3390

Wide variations are possible in the relative proportions of the foodstuffs without injury to health. It is important, however, to consider the limits within which these variations are permissible.

Proteins. From 10 to 15 per cent. of the total Calorie requirements should consist of protein, *i.e.* an average mixed diet with a Calorie value of 3400 will contain about 100 grammes of protein. Where animal protein is entirely absent from the food, a larger amount of vegetable proteins will be necessary, since they are of lower ' biological value ' ; and it is safe to lay down as a rule that for an average man the protein ration should not be diminished below 70 grammes per day. This should include different kinds of protein and at least half of it should be first-class, or animal protein.

Butcher's meat contains from 15 to 20 per cent. protein, and from 15 to 30 per cent. fat. Its importance in the dietary is greater in northern latitudes and the protein of such a diet is considerably more than is required for the mere repair of tissue waste. Owing to the high specific dynamic action of protein, a protein meal raises the production of heat in the body, which is a distinct disadvantage when work has to be

* CHITTENDEN. "Physiological Economy in Nutrition," 1904 (Stokes); "The Nutrition of Man," 1907.

accomplished at a high external temperature. On the other hand, a diet in which there is a large proportion of meat is of value to men in sedentary occupations in a cold or temperate climate. The idea that the heavy worker requires a large supply of meat is of doubtful foundation, though in occupations involving exposure to cold and wet it will add to the comfort of the individual by keeping him warm.

Meat is not only easily presented in a palatable form, but its flavour renders other kinds of foods acceptable. It is thus habit rather than strict physiological principles which will probably govern the quantity of meat regarded as desirable.

Carbohydrates are as a rule the most abundant and cheapest constituents of any diet, and form the greater part of most diets. A certain amount of carbohydrate is essential for man. In the absence of carbohydrate in the food there is a deficient oxidation of the fats, giving rise to acid intoxication.

Fats can be produced by the animal body from carbohydrate, and part of either the fat or the carbohydrate of a normal diet is mutually interchangeable for an isodynamic amount of the other. Although some men can remain healthy on almost fat-free diets provided vitamins are available, some fat is essential, and quantities greater than the minimum amount are desirable. In practice we have to provide for each race a minimum desirable quantity of fat, which will differ in different races and be larger in northern than in southern races. Thus the Japanese soldier is content with 20 grammes of fat daily, while in our country the average man would not be content with less than 75 grammes of fat per day. Seventy-five grammes of fat has a Calorie value of about 680, *i.e.* nearly 25 per cent. of the daily requirements, and we may conclude as a general rule that one-fourth of the total energy of the diet should be supplied in the form of fat.

There are probably three reasons for this craving for fat. In the first place, whereas the greater part of the carbohydrate food is absorbed three hours after food has been eaten, the chief absorption of fat occurs between five and six hours after a meal. On this account individuals deprived of fat get hungry some time before the next meal. The lumbermen of Canada satisfy their huge needs in Calories—6000 to 8000 a day—by a diet in which the fat represents 35 to 40 per cent. of the Calories.

In the second place, weight for weight, fat has more than double the Calorie value of starch and sugar. But the difference in bulk is still greater, since fat is taken in a pure form, whereas most other foods contain a considerable proportion of water.

In the third place, carbohydrates are more subject to fermentative changes in the intestines than fats. Overloading the intestines with carbohydrates leads in many individuals to abnormal production of gases, and general discomfort. We may conclude then that although man can for a time dispense with fat, yet, to develop his full efficiency as a working machine, fat is an essential ingredient of his diet.

We thus see that a dietary to maintain a sedentary man in health must fulfil the following requirements :

(1) It must be palatable. Appetite is an essential factor for the digestion and assimilation of the food, so that good cooking and appropriate flavouring becomes an important condition for the maintenance of health.

(2) It must have a sufficient Calorie value (3400 for the average man).

(3) It must contain protein, fats and carbohydrates. The quantity of each of the first two should be not less than 70 and preferably not less than 100 grammes a day. Of the protein, there should be at least 50 grammes of first-class (*i.e.* animal) protein daily.

(4) It should include a certain proportion of fresh foods, such as green vegetables, meat and eggs, and, in the case of children, milk, in order to supply the necessary vitamins.

(5) It must contain a proper proportion of the salts, especially sodium, calcium, potassium, chlorides and phosphates.

Actual Diets. Statistical inquiries into the food consumption of controlled groups of individuals, *e.g.* soldiers, prisoners, school children,* families, or even of whole nations have given results which lend further support to those obtained from laboratory observations. Thus the population of the United Kingdom in 1911 was about 45¼ million persons : its

* FRIEND. "The Schoolboy" (Heffer, Cambridge), 1935.

man-value, as calculated from the age-distribution, had an average of 0·835, so that the total man-value was about 37¾ million. The Calorie value of all the food grown or imported during 1911 was about 47 billion Calories, which works out at about 1¼ million Calories per man-value per annum or roughly 3400 Calories per day.

As an indication of the great improvement in nutrition in modern times as contrasted with that two centuries ago, the investigations of Friend, Chief Medical Officer of Christ's Hospital School, may be quoted. The requirements of boys of school age are about 2800 Calories. The diets in 1704 and 1929 are given below :

Diet of Schoolboys at Christ's Hospital
(After G. E. Friend)

	In 1704	In 1929
Protein	39 grammes	93 grammes
Carbohydrate	188 ,,	394 ,,
Fat	26 ,,	100 ,,
Calories as beer	730	*Nil*
Total Calories	1900	2930

The study of the dietaries of families of different incomes * yields similar results, and is of great importance in these days of economic stress. Before the Great War a proportion (up to 30 per cent.) of the population, owing to a combination of poverty and ignorance were undernourished, and a still larger proportion suffered from qualitative defects of diet. The following Table from Rowntree illustrates the Calorie deficiency in the poorest classes.

Dietaries of Various Classes in 1900
(After Rowntree †)

		Protein	Calories, daily
In York	I. Wages below 26*s*. weekly	70	2574
	II. Wages over 26*s*. weekly	96	3390
	III. Servant-keeping classes	105	3807
Agricultural Labourers, Midlands		74	2868
Able-bodied paupers, York Workhouse		156	3702
Convicts, hard labour		177	4159

Since the last War more thought has been taken to ensure that the poorest classes do not fall below the border-line of minimum requirements, though it cannot be doubted that there are still many who do, for various reasons, fail to get proper nourishment. In these days of widespread semi-education it should be possible to instruct the masses in the matter of economical purchasing of proper food. As an example of diets which combine cheapness with proper nutritive value we may quote again from the B.M.A. Report. The first is the simplest type of diet for a man, and purchased in the most economical way would cost (in 1933) about 5*s*. per week. It provides no variety however.

To take another example, suppose a family consisting of man, wife, and child aged four. Its man-value, as deduced from the Table on p. 865, would be $1·0 + 0·83 + 0·50 = 2·33$, and an example of constituents for a diet costing (in 1933) about 14*s*. per week for the family is given below.

* Cathcart and Murray. An Inquiry into the Diet of 154 Families of St. Andrews. M.R.C. Report No. 151, 1931, and 165, 1932.

† Rowntree. "Poverty, A Study of Town Life" (cited by Starling, "Feeding of Nations"; and by Drummond, "Lane Lectures" (1934)).

B.M.A. Diet No. 1. Bare Ration. No Variety. Man-value 1

Item.	Quantity.	Protein (grams)	Fat (grams).	Carbohydrate (grams).	Calories.
Corned beef . . .	1 lb.	119·2	84·8	—	1278
Cheese	2 ,,	233·2	317·6	28·2	4022
Margarine . . .	¾ ,,	0·7	288·5	—	2684
Flour *or* . . .	7 ,,	320·6	51 1	2397·5	11,620
Bread	11¼ ,,	(367·9)	(10·1)	(2454·8)	(11,666)
Sugar	1¾ ,,	—	—	793·8	3255
Potatoes . . .	3½ ,,	30·1	0 4	287·4	1306
Tea	¼ ,,	—	—	—	—
Fresh fruit and green vegetables. . .	—	—	—	—	100
Total weekly quantities .	—	703 8	742 4	3506·9	24,265
Daily quantities per man	—	100 5	106 1	501 0	3466

Total first-class protein, 352 4 grammes.
Daily first-class protein, 50 3 grammes.

B.M.A. Diet No. 11. Man, Wife and One Child, aged Three to Six Years. Man-value 2·33

Item	Quantity.	Protein (grains).	Fat (grams).	Carbohydrate (grams).	Calories.
Beef	½ lb.	42 7	41·7	—	563
Minced meat . .	1 ,,	85·3	83·5	—	1126
Bacon	1 ,,	46·7	245·8	—	2478
Corned beef . . .	1 ,,	119·3	84·8	—	1278
Liver . . .	½ ,,	45·2	7·3	10 0	294
Cheese	2 ,,	233 2	317·6	28·2	4022
Milk	10½ pts.	196·8	214·2	285·6	3969
Fish (kipper) . .	¾ lb.	48·0	37·8	—	548
Butter . . .	½ ,,	0 5	188 3	—	1752
Lard (dripping) . .	½ ,,	—	226 8	—	2110
Flour *or* . .	13½ ,,	618 3	98 6	4623·8	22,410
Bread	21½ ,,	(703·1)	(19 4)	(4691 3)	(22,296)
Sugar	2½ ,,	—	—	1134·0	4650
Jam	1 ,,	1 4	—	314·8	1296
Potatoes . . .	7 ,,	60 2	0 7	574·7	2611
Peas (dried) . . .	¾ ,,	69 4	2 0	194·1	1100
Yeast	¼ ,,	—	—	—	—
Tea	½ ,,	—	—	—	—
Oatmeal . . .	1¼ ,,	67·5	48·8	396 9	2358
Rice	½ ,,	13·4	0 9	182 1	810
Treacle . . .	½ ,,	3 7	—	135·9	572
Beans (butter) . .	½ ,,	42·2	1 6	141·1	766
Barley	¼ ,,	7·9	0 9	90·6	412
Fresh fruit and green vegetables. . .	—	—	—	—	300
Total weekly quantities .	—	1701·7	1601·3	8111·8	55,425
Daily quantities per man	—	104·3	98·2	497 4	3398

Total first-class protein, 817·7 grammes.
Daily first-class protein per man, 50·1 grammes.

The conversion of these raw materials into adequate meals is a matter of cookery, but teachers of domestic science have translated these findings into actual cookery recipes enabling a family, *e.g.* of man-value about four, to obtain wholesome and palatable diet at a cost of about 23*s.* per week (in 1933).*

Turning to actual diets, the following results were obtained † by study of the diets of sixty-nine working class families, averaging 3·60 man value per family at Newcastle-upon-Tyne in 1934, as compared with the standard considered desirable :—

	Standard	Mean actual	Range in diets that were sufficient
Calories	3,000	2,960	2,500–3,499
Protein, total, g. . .	80	81·95	70–109
Animal protein, g. . .	37	36 31	30–59
Fats, g.	—	94 55	—
Carbohydrate, g. . .	—	445	—
Calcium, g. . . .	0 68	0 627	0 5–0 999
Phosphorus, g. .	1 32	1 232	—
Iron, mg. . . .	15	15 3	—

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ALCOHOL. When alcohol is taken by man in moderate quantities, up to 10 c.c. per hour, the greater part of it undergoes oxidation, mainly in the liver,‡ and leaves the body as carbon dioxide and water. About 2 per cent. of it, which escapes oxidation, is excreted unaltered by the lungs and kidney. This oxidation of alcohol is a result of true utilisation, since the addition of a certain amount of alcohol to the food does not result in an increased output of carbon dioxide. Alcohol can, in this way, act as a food and supply up to 40 per cent. of the daily calories, provided it is taken in small doses, often repeated, so as not to result in there being a high alcohol content in the blood (E. Mellanby).§ The chief effects of alcohol are on the nervous system. The latent period of responses is increased and the intensity of conditioned reflexes is reduced, while the normal balance between excitatory and inhibitory states is upset.‖¶ Its value in a diet is chiefly that of an accessory or adjuvant, in exciting appetite by its taste and smell. When taken in large doses, this function is overshadowed by the poisonous action of the substance. A man unaccustomed to its action cannot take more than 16 to 25 grammes

* "Family Meals and Catering." (B.M.A., 1934. Price 6*d.*)

† A Study of the Diets of Sixty-nine Working Class Families in Newcastle-upon-Tyne [City and County of Newcastle-upon-Tyne]. (*Christie, Malcolm Ltd., Newcastle*)

‡ Lundsgaard. *C. R Lab. Carlsberg*, 1937, **22,** 333.

§ **M.R.C. Special Report, No. 31.**

‖ Andreyev. *Arch. Internat. de Pharm. et de Thérap.*, 1934, **48,** 117.

¶ Horsley Gantt. *Bull. Johns Hopk. Hosp.*, 1935, **56,** 61.

without experiencing its poisonous effects,* yet this amount corresponds to a concentration well below 0·1 per cent. of alcohol in the blood. The blood alcohol reaches a peak about one and a half hours after the consumption, and after 96 c.c. alcohol (about 8 oz. of 40 per cent. whisky) the peak value in blood is about 0·2 per cent. A blood alcohol content of 0·15 per cent. usually causes mild intoxication, and 0·25 per cent. definite drunkenness, though in some habituated individuals concentrations of well over 0·2 per cent. can be borne with little apparent effect. The rate at which alcohol is oxidised is accelerated by habituation.† The rate of absorption of alcohol into the blood determines the speed with which it acts, and depends upon the rate of emptying of the stomach. Hence the taking of food, and particularly of

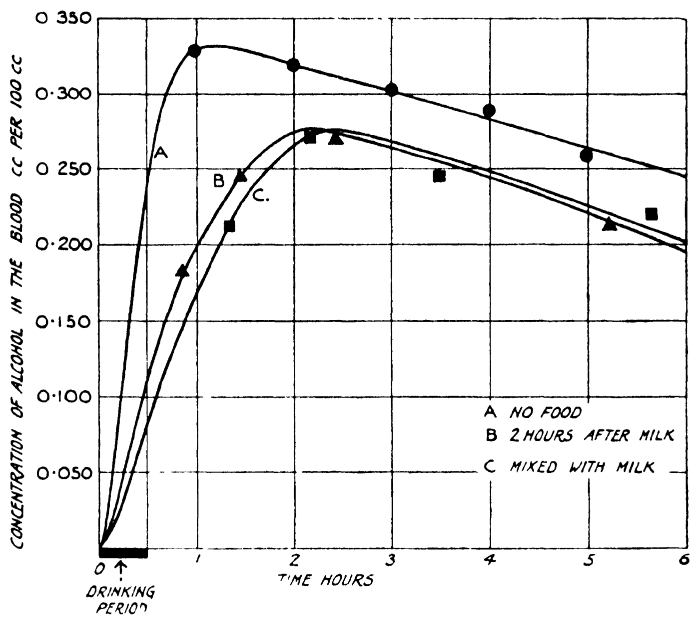


FIG. 526. A. Concentration of alcohol in the blood of a man after taking 171 c.c. absolute alcohol in the form of whisky. B. The same dose preceded by 1 pint of milk. C. " The same again," accompanied by a pint of milk. (After MELLANBY.)

fat, delays the absorption of alcohol (Fig. 526) so that alcohol taken on an empty stomach will produce the speediest and greatest effects.

The removal of alcohol from the blood by excretion and oxidation takes place, according to Mellanby, at a constant rate of about 0·012 per cent. per hour, so that it takes ten hours to get entirely free from alcohol after it has reached a concentration of 0·12 per cent. in the blood. Haggard and Greenberg,‡ however, believe that the rate of oxidation is proportional to the amount of alcohol present in the body. Alcohol is excreted in the breath and in the urine (Fig. 527), the concentration of alcohol in both depending on its concentration in the blood : the concentration in the urine is 1·35 $\times$ that in the blood. It is stated that 0·36 per cent. of alcohol in the urine corresponds to a definite state of drunkenness. In any case, the alcohol

* M.R.C. Special Report, No. 34 (Vernon), and 56 (McDougall and Smith).
† TURNER. *J. Pharmacol. Exp. Ther.*, 1932, **44**, 305.
‡ HAGGARD and GREENBERG. *J. Pharmacol. Exp. Therap.*, 1934, **52**, 167

concentration in the urine is so small that, if excretion by the kidney were solely responsible for the removal of alcohol from the body, it would take about a month to remove 100 c.c. of alcohol from the body.* As regards

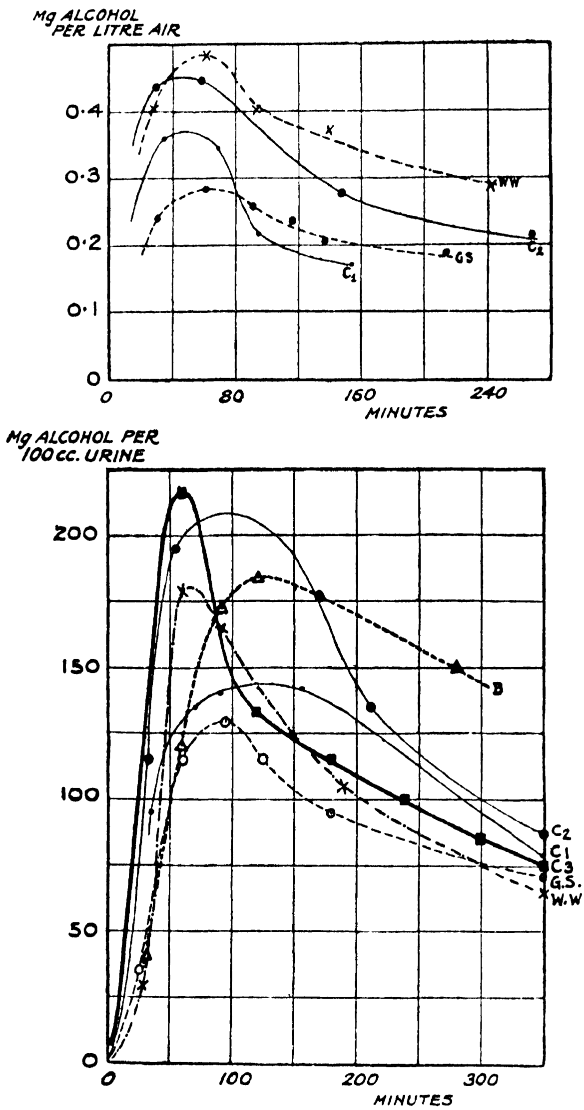


FIG. 527. Concentrations of alcohol in breath (upper curve) and in urine (lower curves) of different individuals, after taking 86 c.c. of absolute alcohol (in the form of 216 c.c. whisky). (SMITH and STEWART, *B.M.J.*, 1932 (I), 87.)

removal by the breath, this is about twice as rapid as by the kidneys, according to the results shown in Fig. 527, since each minute about 8 litres of air, containing about 3 to 4 mg. of alcohol, leave the body, *i.e.* about 6 grammes per day.

* HAGGARD and GREENBERG. *J. Pharmacol. Exp. Therap.*, 1934, **52**, 150.

CHAPTER XLIV

THE PHYSIOLOGY OF DIGESTION

CHANGES UNDERGONE BY THE FOODSTUFFS IN THE ALIMENTARY CANAL

In most cases the foodstuffs as we ingest them are colloidal, neutral, and insoluble. When we take these colloidal or insoluble substances into our alimentary canal, they have to be rendered soluble and diffusible, in order to allow of their easy transference across the wall of the gut into the blood. The cells of the body cannot deal with all kinds of carbohydrate. Most animal cells will starve when presented with starch or disaccharide, such as cane sugar. It is necessary, therefore, that all the carbohydrates shall be altered to the form of monosaccharides. As regards proteins, the change must be even more profound. To form serum albumin, for instance, out of wheat gliadin, an entire reconstruction is necessary. This can only be accomplished by taking the protein molecule to bits, selecting certain of its constituent parts and building these up in the proper proportions to make a new protein molecule. This process of resynthesis must be carried out in the tissue cells themselves.

In all the higher animals the solubilisation of the food is accomplished in the lumen of the alimentary canal by means of *digestive juices*, formed by the activity of glands produced as outgrowths from the wall of the canal. The digestive juices attack the foodstuffs by means of enzymes, and the action of these is hydrolytic. Since each class of foodstuff requires different enzymes, a great variety of these is concerned in the process of digestion.

As the end result of digestion the many kinds of food are reduced to a fairly small number of simple substances. These end-products are :

(1) *Carbohydrates.* The monosaccharides: glucose, fructose, and galactose.

(2) *Fats.* Fatty acids and glycerol.

(3) *Proteins.* A great variety of mono- and diamino-acids.

Those constituents of the food, such as the water and salts, which undergo no metabolic alteration in the body, pass along the alimentary canal practically unchanged; if absorbed they pass without further alteration into the blood.

1. DIGESTION IN THE MOUTH

It is common experience that, when food is taken, there is a flow of 'saliva' into the mouth. Saliva is the product of secretion from three pairs of large salivary glands situated in the neighbourhood of the mouth and pouring their secretions into it by means of ducts. It is possible to collect the fluid secreted by each of these glands separately; and it is found that the saliva varies in properties according to the gland from which it is derived. In addition to these large glands the whole mucous membrane of the mouth is beset with small glands, the buccal glands.

The saliva is in most cases a mixture of the secretions of all three pairs

of salivary glands as well as of the small glands of the mucous membrane. It forms a colourless cloudy liquid, slimy in character. The cloudiness is due to the presence of a number of desquamated epithelial cells, lymphocytes, as well as colloidal clumps of mucin. Its reaction as secreted is neutral, or very nearly so (pH 6·4—7·0), and it is well buffered. On standing or heating it loses CO_2 and becomes alkaline and turbid from deposition of calcium phosphate, which may be deposited as 'tartar' on the teeth.* Its specific gravity varies between 1002 and 1008 and freezing point $-0{\cdot}07$ to $-0{\cdot}34°$ C. Its chief constituents are mucin, and in some cases a diastatic enzyme, ptyalin, and traces of coagulable proteins, potassium thiocyanate, urea, &c. Its approximate composition in man is as follows :

Water	99·27	Na	0·02
Solids	0·73	K	0·06
Organic Solids	0·50	Ca	0·01
Mucin	0·30	CNS	0·03
Inorganic Solids	0·23	HCO_3	0·10
Cl	0·05	Total N.	0·07
P.	0·02		

Gases are about 1 c.c. O_2, 2·5 c.c. N_2 and 50 c.c. CO_2 per 100 c.c.

Potassium thiocyanate is formed in the body whenever cyanides or organic nitriles make their appearance, either as the result of administration or as by-products in the normal processes of metabolism. The conversion of the poisonous cyanides into the almost innocuous thiocyanates seems to be a means by which the organism protects itself against the poisonous effects of the former. Saliva of smokers contains more thiocyanate than that of non-smokers. The channels of excretion of the thiocyanate are by the salivary glands, the kidneys, and the gastric juice.

The Uses of Saliva. The main function of saliva is to moisten the food and so facilitate its mastication and deglutition. The presence of the mucin is of special value for the latter process, since it renders the mass of food slippery. In animals such as dogs, where the saliva is devoid of any digestive enzyme, this must represent its sole function.

In man and some of the herbivora the saliva contains an enzyme, called *salivary amylase*, or *ptyalin*, which exerts a digestive effect on starch. If a warm solution of starch be taken into the mouth, kept there for one minute, and then expelled into a test-tube, the starch will be found to have entirely disappeared, its place being taken by a reducing sugar. The stages in the action of saliva on boiled starch can be followed more easily when a very small amount of saliva is added to some starch solution at 37°, and portions of the digest are tested at intervals. The first change is a conversion of the opalescent gelatinising starch solution into a clear solution which no longer sets on cooling, but still gives a blue colour with iodine. This is known as soluble starch. The soluble starch then undergoes hydrolytic dissociation into a dextrin, which gives a red colour with the iodine and is therefore known as erythrodextrin, together with maltose. The erythrodextrin is then hydrolysed into an achroödextrin (giving no colour with iodine) and maltose, and the achroödextrin is still further broken up into stable dextrin and maltose. The conversion of starch into maltose by ptyalin is practically arrested when 80 per cent. of the starch has been so converted, the remaining 20 per cent., called stable dextrin, being resistant.† The stages in the conversion are represented in the following Table :

* PHILLIPS. *Ztschr. f. physiol. Chem.*, 1935, **233**, 208.
† LOVATT EVANS. *Journ. Physiol.*, 1912, **44**, 191.

Starch

Soluble Starch

(Erythro-) Dextrins — Maltose

(Achroö-) Dextrins — Maltose

Stable Dextrins — Maltose

The process by which the huge starch molecule is converted into dextrins and maltose is a very complicated one, and a number of intermediate compounds of dextrins and maltose may exist between those whose presence is revealed by their varying reaction to iodine. On very prolonged action of saliva, some of the maltose formed is further hydrolysed to glucose, but this action is too slow to be of any importance in the body.

Ptyalin is most active at a *p*H of about 6·0, so that the addition of minute traces of acid to an alkaline saliva increases its activity. Further acidification, however, rapidly destroys it. Removal of salts by dialysis renders it almost or quite inactive, the activity returning when the salts are restored. Addition of small amounts of chlorides greatly accelerates the action of saliva. It acts most rapidly at 45°, but even at 0° C. its action is still just perceptible. If heated to 60° C. it is destroyed.

When the starch is in the solid condition, as in biscuits and most farinaceous foods, its stay in the mouth during the normal process of mastication is not long enough to allow of any considerable hydrolysis occurring. When a meal is taken, the food which is swallowed forms a mass which is penetrated only slowly by the acid gastric juice secreted by the stomach within five minutes of the taking of food. Even half an hour after a meal the interior of the mass of food in the stomach may be still found to be neutral or slightly alkaline. The food, therefore, thoroughly moistened by and mixed with saliva, remains in the stomach for thirty to forty minutes, gradually reaching its optimum *p*H, and with chloride accumulating and also accelerating its action, before the amylase is destroyed by the acid gastric juice. During this time the ptyalin continues to exert its effect, so that we may say that the chief part of the salivary digestion occurs actually in the stomach, and results in an almost complete conversion of the starch into dextrins and maltose. Then the food becomes thoroughly soaked with the acid gastric juice, and salivary digestion ends.

THE SECRETION OF SALIVA

The greater part of the saliva is formed in man by the sublingual and the submaxillary glands.

The arrangement of these glands, especially of those in the floor of the mouth, varies somewhat in different animals. They can be divided, according to their structure and the nature of their secretion, into three classes. Among the small buccal glands we may distinguish two types, the *mucous gland* and the *serous gland*. The mucous gland is distinguished by the fact that its short duct opens into wide alveoli, the lining cells of which are distended with mucin and therefore present a clear unstained space in the section. In the other type, the serous gland, the duct lined with columnar cells branches into a series of acini, which present a well-marked lumen and are lined with small

granular cells with a very distinct and well-staining nucleus. The same general distinction can be made out in the large salivary glands. The parotid gland in man and in all the higher mammalia is a typical serous gland, though here and there a mucous cell may be seen. The sublingual and submaxillary glands in man represent a third type called *mixed* glands. Most of the alveoli are mucous in character. At the ends of the alveoli are seen crescent-shaped serous cells between the mucin-distended cells and the basement membrane. These are known as the *demilune cells*, or the *Crescent cells* of Gianuzzi.

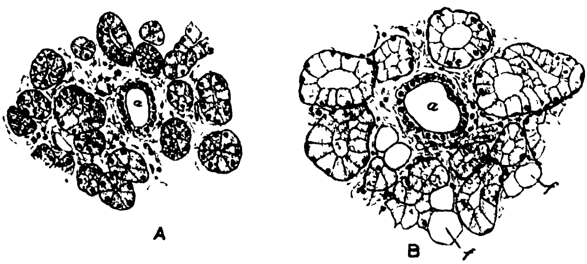


FIG. 528. A. Serous Gland. B. Pure Mucous Gland from Mouth. (KOLLIKER.)
a. Ducts. *f.* Fat cells.

In some cases these mucous alveloi with demilunes may be found alongside of typical serous alveoli.

Thus in man the submaxillary gland is usually a mixed gland, the serous alveoli predominating. The sublingual gland is also mixed, but with a predominance of the mucous alveoli. In the monkey the submaxillary gland is almost entirely serous. In the dog the submaxillary gland is a mucous gland with some demilunes, while the retrolingual and sublingual glands when present are of the mixed type. In the rabbit the submaxillary gland is serous, while the sublingual gland is mucous. In the cat the submaxillary is mucous, the retrolingual is mixed, and the sublingual, when present, is mixed, with predominance of the mucous type.

The secretion of the separate glands in man can be studied by passing a fine catheter into the appropriate duct, and also in certain rare cases where fistulæ occur in the parotid duct. The normal behaviour of the salivary glands during digestion is best studied by aid of a method used long ago by de Graaf and reintroduced by Pavlov. It is possible, without any disturbance of the animal's nutrition, to transplant the papilla, on which the duct opens, to the outside, so that the saliva from any particular gland shall flow externally instead of into the cavity of the mouth. These fistulated glands retain their normal structure and functions for many months, though the gland finally becomes fibrotic. By attaching a small funnel to the fistulous opening, it is easy to collect the pure saliva.

In man the glands are continually secreting slowly * ; in carnivora there is normally no secretion when no stimuli such as food are present ; as soon as food is introduced into the mouth there is a rapid secretion of saliva, the relative extent to which different glands are involved varying according to the nature of the stimulation. Thus with meat and other moist foods there is (in the dog) only a small amount of secretion, which is derived chiefly from the submaxillary and sublingual glands, and is rich in organic constituents. When dry or undesirable material, such as dry powdered meat, or sand, is introduced, the flow of juice is more copious and more watery. Secretion may be produced reflexly in the dog by showing food, or even by the suggestion that dry powder is to be introduced into the mouth. This is an example of a conditioned reflex.

The serous and mucous glands differ in the nature of their secretion. A

* LASHLEY. *J. exp. Psychol.*, 1916, 1, 461.

serous gland, such as the parotid, gives a clear watery secretion almost free from mucin, but containing small traces of coagulable protein. The mucous gland delivers a secretion which is viscid from the presence of mucin, and contains also a small trace of coagulable protein. Both in parotid and mucous saliva the percentage of salts is very low. In the dog the saliva is free from any enzymes. In man amylase is found in the saliva from both kinds of glands, though it is about four times as concentrated in that secreted by the parotid gland. The total amount of saliva which may be obtained varies of course in different animals. Each gland may, however, in the course of the day give an amount of juice far exceeding, *e.g.* ten or twelve times, its own weight. In man it is reckoned that over one litre of saliva may be formed every twenty-four hours, and in the herbivora such as the horse, the total diurnal production must amount to many litres. In thirsting the reflex secretion of saliva is reduced

Nervous Arrangements of Secretion. The activity of the salivary glands must be excited by reflex means. The *afferent nerves* in this reflex include those that supply the mucous membrane of the mouth, *i.e.* the fifth nerve, the pharyngeal branch of the vagus, and the glossopharyngeal.

The salivary 'centre' consists of a neurone pool in the recticular formation extending from the sensory nucleus of the VIIth cranial nerve down to the anterior end of the nucleus ambiguus; the more caudal part supplies the parotid, the rest the other two glands. Certain areas of the cerebral cortex also give salivation when excited, but the significance of this is doubtful.

Sympathetic subordinate centres are located in the spinal cord, and are probably under superior control from the hypothalamus.

Each one of the large salivary glands receives efferent nerve fibres from two sources: from the parasympathetic and from the sympathetic system.

The *parasympathetic fibres* to the submaxillary, the sublingual, and the retrolingual glands pass into the facial nerve, and from this nerve along the chorda tympani to the lingual division of the fifth nerve.* The lingual nerve passes below the duct, and just before it crosses the two ducts of the submaxillary and retro- or sub-lingual glands, it gives off a small branch backwards, the *chorda tympani*, which runs along the submaxillary duct to be distributed to the glands, and in its course gives off fibres also to the retrolingual (Fig. 529). Two neurones are involved in the cranial supply. By means of the nicotine method, Langley showed † that all the preganglionic fibres to the sublingual and the submaxillary glands end somewhere near the glands in connection with ganglion cells, from which non-medullated post-ganglionic fibres pass to the glands. These relay ganglia for the submaxillary gland are scattered cells lying in the hilum of the gland itself; in the cat those for the retrolingual gland are in the so-called 'submaxillary ganglion.' The fibres to the sublingual gland in man probably take a similar course. The fibres are apparently finally distributed to the secreting alveoli, where they end in the mucous secreting cells.‡

The parasympathetic fibres to the parotid gland leave the medulla by the glossopharyngeal nerve and run in its tympanic branch (nerve of Jacobson) to the tympanic plexus, and then by the small superficial petrosal nerve to the otic ganglion, where relays occur, the post-ganglionic fibres then passing

* LUDWIG. *Ztschr. f. rat. Med.*, 1851, N.F. 255; CLAUDE BERNARD, *Syst. Nerveux*, 1858, II., 150.

† LANGLEY. *Journ. Physiol.*, 1890, **11**, 123.

‡ RAWLINSON *Anat. Rec.*, 1933, **57**, 289

to the gland by the auriculo-temporal branch of the third division of the fifth nerve.* The site of termination in the gland is doubtful.

The *sympathetic supply* to all these glands is contained in fine filaments

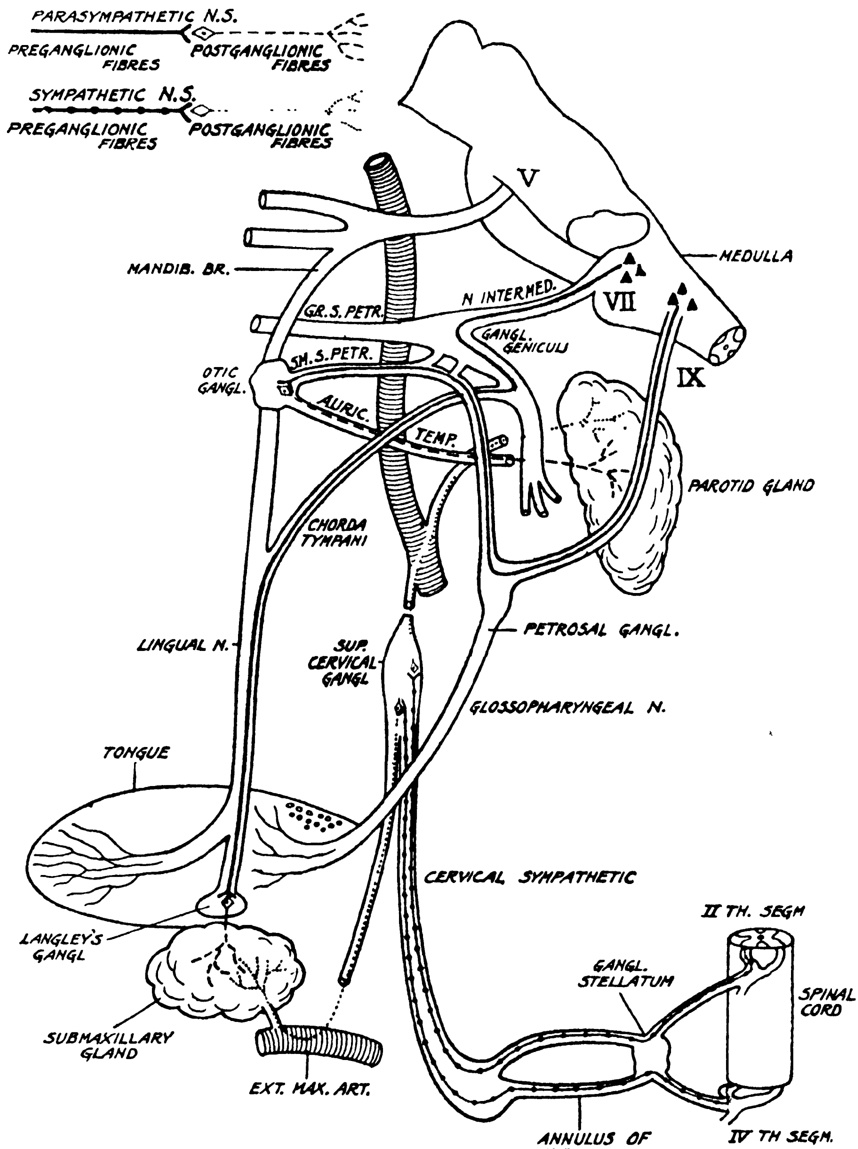


FIG. 529. Diagram of the efferent nerves to the submaxillary and parotid glands. (Modified from BABKIN after MÜLLER.)

on the walls of the arteries with which they are supplied. The fibres are derived from the spinal cord, whence they issue by the upper three or four ventral thoracic nerve roots. They pass into the cervical sympathetic as

* CLAUDE BERNARD. *Leçons sur la physiol. et la pathol. du système nerveux*, Paris, 1858 II., p. 158.

preganglionic fibres which end around the cells of the superior cervical ganglion, and fresh relays of fibres, chiefly non-medullated, arise from these cells and travel on the walls of the branches of the external carotid artery to their destination, which is in the demilune cells of mixed glands, and the main cells of serous glands.

The effects of stimulating the peripheral ends of the parasympathetic nerves going to the glands present a general resemblance, whichever be the gland involved.* Within a period of half to two seconds after the stimulation has been applied, a secretion of saliva is produced, presenting similar characters to that which would be obtained from the gland under normal conditions. The concentration of the saliva, as well as its rate of secretion, depends on the strength of the stimulus. The following table (Heidenhain) shows the effect on the amount and composition of submaxillary saliva obtained by weak and strong stimulation of the chorda tympani nerve :

Strength of stimulus.	Quantity in one minute.	Per cent. of organic solids.	Per cent. of salts.
Weak . . .	0·17	0·84	0·20
Strong . . .	0·72	2·06	0·46
Weak . . .	0·17	1·67	0·26

With the strong stimulus the amount of saliva was increased over four-fold, while the percentage of organic substances in the saliva was raised from 0·84 to 2·06 per cent. There was at the same time an increase in the percentage of salts. If the excitation be continued for a considerable time, there is a gradual rise in the percentage of inorganic salts and a fall in the percentage of organic matter.

The cranial nerves going to these glands produce another important effect, namely vaso-dilatation. It was shown by Claude Bernard that on exciting the chorda tympani the flow from the vein of the submaxillary gland might increase four to eight times, and indeed to such an extent that, in spite of the fact that the secreting gland has a greatly increased oxygen utilisation,† the blood passing through the gland did not stay long enough to lose much oxygen. As the result of exciting the chorda, therefore, the blood coming from the vein may show distinct pulsation, and may have a brilliant scarlet hue just as if it were derived from an artery. The same dilatation has been observed to attend excitation of the cranial supply to the parotid gland.

It is probable that all the effects of stimulation of the parasympathetic nerve supply upon a salivary gland are due to the liberation at the nerve terminations ‡ of small amounts of acetyl choline, the injection of which into the blood stream produces effects similar to those of the nerve stimulation.

Pilocarpine also causes profuse salivation, but the saliva produced differs in composition from that resulting from chorda stimulation.

The effects of exciting the sympathetic nerve supply differ according to the gland and the animal. In the dog, excitation of the cervical sympathetic causes the secretion of a few drops of thick viscid saliva from the submaxillary gland. In this animal no secretion is obtained at all from the parotid gland on exciting the sympathetic, though some result of stimulation is shown by the occurrence of histological changes in the gland cells. In the cat, the

* Heidenhain. *Pflüger's Arch.*, 1878, **17**, 1.
† J. Barcroft. *Journ. Physiol.*, 1901, **27**, 31
‡ Babkin, Gibbs and Wolff, *Arch. f. exp. Path.*, 1932, **168**, 32 ; Gibbs and Szelöczey *ibid.*, p. 64.

submaxillary saliva obtained on sympathetic excitation may be as copious as that obtained on stimulation of the chorda tympani. We shall have later on to discuss how far these results are to be ascribed to a fundamental difference in the effects of impulses carried to the secreting cells by the two sets of nerve fibres, and how far to their different effects on the blood vessels. It must be remembered that the sympathetic supply carries the vasoconstrictor fibres to most or all of the vessels of the head and neck, and therefore stimulation of this nerve causes vascular constriction in the gland affected.

A motor effect, producing a shrinkage of the gland, is also seen in all the salivary glands on stimulation of the sympathetic supply. It is caused by the contraction of special myo-epithelial cells lying just inside the basement membrane.*

Changes in the Gland Accompanying Secretion

The fact that a submaxillary gland of the dog under favourable conditions will secrete its own weight of saliva in five minutes, and will on continued stimulation secrete for many hours afterwards, shows that there is a continual renewal of the fluid which is turned out in the secretion. If we refer to the diagrammatic representation of the elements which make up a secreting lobule and which may be involved in the act of secretion (Fig. 530), we see that between the lumen of the duct and the blood, which must be regarded as the source of the fluid, the following layers of cells intervene: (1) the endothelium of the blood capillaries; (2) the basement membrane; (3) the epithelial cells of the gland proper. We have in the first place to decide to which of these elements can be ascribed the chief part in the act of secretion.

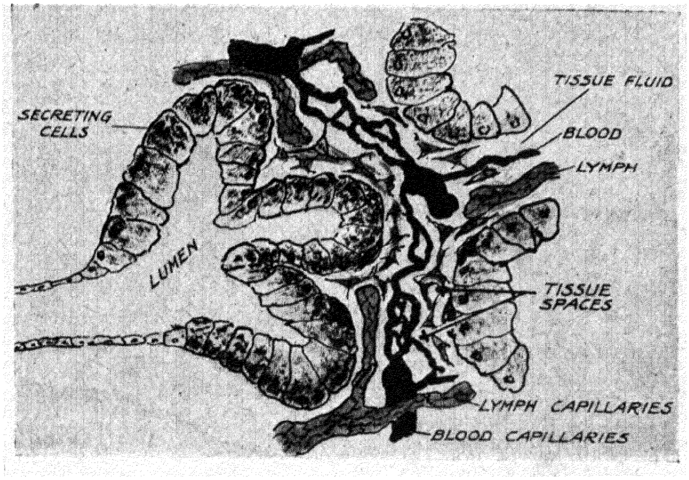
The secretory activity of the submaxillary gland is usually associated with a considerable dilatation of the vessels of the gland, and a consequent large increase in the blood flow through the gland, which may amount to between three and eight times the quantity passing during rest. Such an increase in the supply of blood affords a source for the large quantity of fluid which is turned out in the saliva and raises the pressure in the capillaries of the gland. We cannot, however, regard this rise of pressure as the essential factor in the act of secretion. If atropine be administered, excitation of the chorda causes the same vaso-dilatation as before, though no secretion is produced. Moreover, Ludwig showed that the force with which the secretion is turned out into the ducts of the gland is greater than that represented by the blood pressure. If two manometers be connected, one with the carotid artery and the other with the duct, on stimulating the chorda tympani nerve the mercury will be driven up by the pressure of the secretion in the corresponding manometer until it attains a height which may be double that of the mercury in the manometer connected with the carotid artery,† and therefore must be still greater than the pressure in the capillaries of the gland. This experiment showed the impossibility of the act of secretion being a process of filtration.

We have now further evidence that work is done in the production of the salivary secretion. When a fluid containing salts in solution is filtered through a porous membrane, the filtrate has the same content in salts as the original fluid. We can effect a separation of dissolved salts from a fluid by filtration under pressure through a semipermeable membrane. Under these circumstances a very large pressure is necessary in order to cause the

* BABKIN and MACKAY. *Amer. J. Physiol*, 1930, **91**, 370.

† LUDWIG. *Mitt. d. Zürch. Naturf.*, **1851**, Nr. 50, 271. (Ostwald's *Klassiker d. Exakten, Wiss*, Nr. 18.) This effect is only well seen if the arterial pressure is low.

filtration of any fluid at all, a pressure greater than the osmotic pressure exerted by the substances in solution. Thus if we were filtering a 1 per cent. solution of NaCl through a semi-permeable membrane, we should have to exert a pressure of about seven atmospheres in order to obtain a filtrate free from sodium chloride. To obtain a filtrate containing half the amount of sodium chloride, if such were possible, would therefore need a pressure of



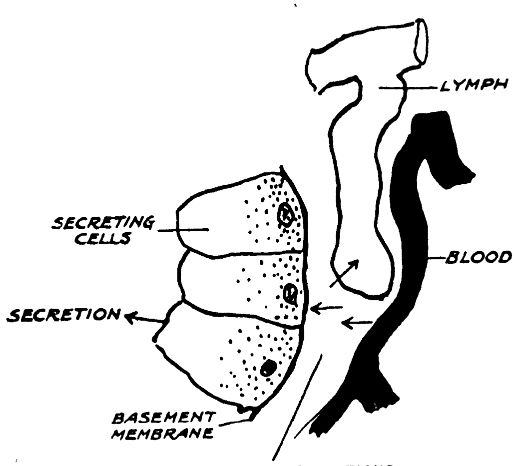


FIG. 530. Diagrams to show Relation of the Secreting Cells of a Gland to the Blood and Tissue fluid and Lymph Drainage. The lower figure is schematic.

about three and a half atmospheres. On comparing the osmotic pressures of saliva and blood respectively—using the depression of freezing-point as our index—we find that the osmotic pressure of saliva is between half and three-quarters that of the blood plasma. Supposing the membrane separating the lumen of the duct from the blood vessels could be regarded as a semi-permeable membrane, we should need, in order to effect the separation, a pressure ten to twenty times as large as the arterial blood pressure. We must conclude, then, that work, both osmotic and mechanical, is performed in the separation of the fluid from the blood and its transference in the form of saliva to the duct.

A simple experiment shows that this work must be effected by the gland cells themselves. The fluid passes from the blood vessels first into the tissue spaces, whence it is taken up by the secretory cells. If the first act in secretion consisted in an increased exudation of fluid from the blood into the tissue spaces, the first effect of exciting the chorda tympani nerve should be to cause the swelling of the whole gland. By placing the gland in a plethysmograph we can record the actual changes in its volume which ensue on excitation of the chorda tympani.* If all secretion be prevented by the previous administration of atropine, stimulation of this nerve produces, as might be anticipated, an increased volume of the gland in consequence of the dilatation of its vessels. If, however, the gland be allowed to secrete, we obtain, in spite of the simultaneous increase in size of the vessels, an actual diminution in the size of the gland itself, showing that the first effect of the stimulation is on the cells of the alveoli (Fig. 531). Under the stimulus, these empty themselves of the fluid they contain, replenishing themselves at the expense of the fluid in the tissue spaces. The increased passage of fluid from blood to tissue space is therefore a secondary and not a primary effect of the nerve stimulation.

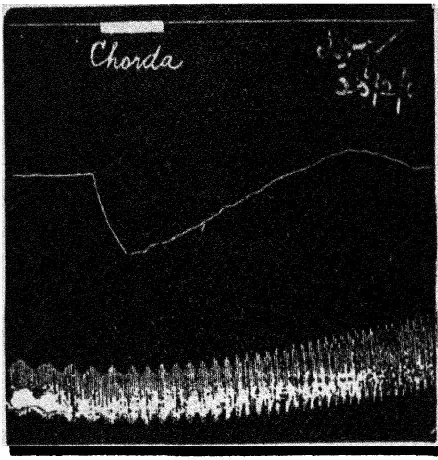


FIG. 531. Tracing of Volume of Submaxillary Gland, showing Decrease on Excitation of Chorda. (BUNCH.)

HISTOLOGICAL CHANGES DURING SECRETION. The process of secretion is associated with changes in the structure of the secretory cells. The changes are of the same general character whatever class of glands we investigate, though the ease with which they are to be demonstrated varies with the effects of the hardening fluids usually employed. If a small fragment of a mucous gland be teased in blood serum or in 2 per cent. NaCl solution, the cells are found to be packed with a mass of coarse, highly refractive granules (Fig. 532). If a corresponding specimen be made from a serous gland (Fig. 533), the cells are also packed with granules which, however, are much finer in structure. On making similar specimens from glands which have been forced to secrete for six or seven hours, the individual cells are found to be much smaller and the protoplasm of the cell is relatively increased in amount, while the granules are much fewer and are now confined almost entirely to the inner margin of the cell.† Activity is thus associated certainly with a discharge of granules, and probably with some increased building up of protoplasm. We may regard the act of secretion as determined by the alteration of the granules and their discharge, together with water and salts, to form the specific secretion of the gland. During rest the granules are re-formed by precipitation in, or modification of, the protoplasm surrounding the nucleus. We have evidence that, although the granules form the secretion, they represent, not the secretion itself, but a precursor of some at any rate of its constituents. Thus if acetic acid be added to the saliva obtained from the submaxillary gland, the mucin is precipitated as threads and films. If the granules in the secreting cells also consists of mucin, we should expect acetic acid to have a coagulating effect upon them. We find on the contrary that, on allowing acetic acid to flow over a section of the fresh gland, the granules at once swell up and burst. We must regard these granules therefore, not as mucin, but as a precusor of mucin, mucinogen. The effect of ordinary fixing reagents is to cause these granules to swell up so

* BUNCH. *J. Physiol.*, 1900, **26**, 1.
† LANGLEY. *Journ. Physiol.*, 1879, **2**, 261.

that the cells become filled with a mass of mucin giving the typical hyaline appearance of ordinary sections of these glands. In the case of the serous glands the granules

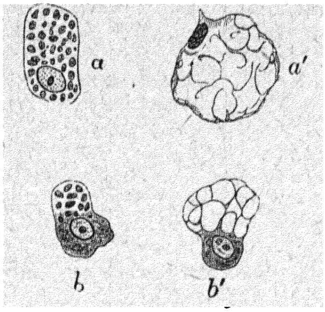


FIG. 532. Mucous Cells from a Fresh Submaxillary Gland of a Dog. (LANGLEY.)

a. Mucous cell examined fresh from a resting gland.

a′. The same cell treated with weak alcohol.

b and *b′*. Cells from a discharged gland before and after treatment with weak alcohol.

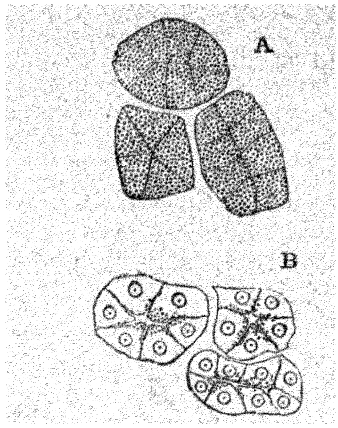


FIG. 533. Acini of a Serous Salivary Gland. (LANGLEY.)

A. Resting condition.

B. Discharged condition.

(Fig. 534) are apparently protein in nature. Where ptyalin is a constituent of the saliva, we are probably justified in assuming that it is contained as a precursor in the granules. It is customary to speak of the granules in a secreting gland as *zymogen* granules, *i.e.* the precursors of enzymes. It is probable that we ought to regard these granules, not merely as precursors of the constituents of the secretion, but as cell organs in which proceed a whole series of chemical and osmotic changes, which determine the production of the fully formed secretion directly from the protoplasm and indirectly from the ordinary constituents of the surrounding tissue fluid.

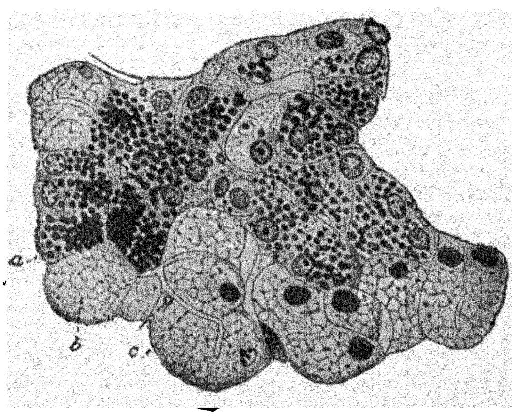


FIG. 534. Submaxillary Gland of Rabbit. (SCHAFER after E. MULLER.)

The cells, all serous, are in different functional states:
a. A loaded cell.
b. A discharged cell.
c. A secretory canaliculus penetrating into a cell.

ELECTRICAL CHANGES. The interpretation of the electric potential changes which accompany secretion is difficult, owing to the simultaneous effect of the movement of fluids through porous membranes. If the hilum of the submaxillary gland and its outer surface be connected with a galvanometer, the outer surface is positive to the hilum. On exciting the chorda tympani nerve a diphasic effect is generally obtained, the resting difference being first increased and later on diminished. On excitation of the sympathetic nerve we generally obtain a purely negative variation of the resting difference. These results were interpreted by Bayliss and Bradford * as due to the co-operation of the two factors, chemical change in the gland cells and

* BAYLISS and BRADFORD. *J. Physiol.*, 1885, **6**, xiii; 1886, **7**, 217.

movement of fluid through the cells. The positive variation, *i.e.* the current from within out, was ascribed to the movement of fluid, whereas the negative variation of the resting difference was thought to be due to the chemical changes in the gland cells.

The Significance of the Double Nerve Supply to the Glands

Heidenhain * claimed that stimulation of the sympathetic supply to a salivary gland causes histological changes in the gland even more marked than those produced by the cranial nerve. He therefore put forward the hypothesis that the salivary glands are supplied by two fundamentally different classes of fibres, namely (1) trophic fibres, which are responsible for the production of the specific constituents of the secretion, and (2) secretory fibres, excitation of which causes the cells to take up water and salts from the tissue fluid and blood, and pass them in large quantities into the duct. The sympathetic supply would consist almost entirely of trophic fibres, whereas secretory fibres would predominate in the parasympathetic supply.

According to Langley† and Carlson, the difference in the action of the chorda tympani and of the sympathetic on the submaxillary gland is due to the opposite action of these nerves on the blood supply to the gland, the sympathetic causing vaso-constriction, and the chorda tympani vaso-dilatation; they find that clamping the carotid artery during chorda stimulation diminishes the amount of saliva secreted but increases the percentage of solids in the fluid. Although this theory may provide a partial explanation, it is inadequate to explain the differences observed in the secretion of saliva reflexly aroused by introduction of substances into the mouth. In a dog with a permanent submaxillary fistula a copious flow of saliva may be caused by the introduction of 0 25 per cent hydrochloric acid or of meat powder. The amount of saliva secreted under the two circumstances is approximately the same, but that evoked by the introduction of meat powder contains about five times as much organic solids as that which follows the introduction of acid into the mouth. The clue to the variation is probably to be sought in the different effects of sympathetic and parasympathetic fibres (or of their chemical representatives adrenaline and acetyl chlorine) upon different cells of the salivary glands. Stimulation of the chorda in the cat, for instance, causes profound histological changes in the alveolar cells of the submaxillary gland, while sympathetic stimulation, leaving those unaffected, causes changes in the demilune cells.‡ The composition of the salivas obtained in the two cases is characteristic of the secretions of the mucous and serous cells respectively.§

Even this explanation may not be complete; Babkin has shown that variation in the saliva is found after complete section of the sympathetic and that there is the same acceleration of the circulation through the gland whether the secretion is aroused by introduction of meat powder or of acid. It is impossible, therefore, to explain the difference in the composition of the saliva obtained under these two circumstances as due solely to differences in the blood supply to the gland, and we must conclude either that the chorda tympani contains different kinds of fibres which are excited to varying extents according to the nature of the reflex stimulation, or that one and the same nerve fibre can convey specifically different impulses. At any rate, it is certain that, according to the nature of the reflex stimulus, either the secretion of water and salts or the secretion of organic solids may preponderate, altogether apart from changes in the circulation simultaneously evoked. It has also been found that simultaneous stimulation of the chorda tympani and sympathetic nerves gives rise to an *augmented secretion* of saliva, greater than that given by the chorda alone, although the blood flow through the gland is less. (GESELL.)

The Energy Involved in the Act of Secretion

Barcroft ‖ attempted to determine the total amount of energy set free by the gland in secretion, by measuring its respiratory exchange under conditions of rest and activity. He found that the resting submaxillary gland in a small dog took up 0·25 c.c. of oxygen per minute, while during active secretion it absorbed 0·86 c.c. O_2. Assuming that the total oxygen taken up is employed in the oxidation of a food substance, such as glucose,

* HEIDENHAIN. *Pflüger's Arch.*, 1878, **17**, 1.
† LANGLEY. *J. Physiol.*, 1878. **1**, **96**.
‡ RAWLINSON. *Anat. Rec.*, 1933, **57**, 289.
§ LANGSTROTH, MCRAE and STAVRAKY. *Arch. internat. pharmac.*, 1938, **58**, 61.
‖ BARCROFT. *J. Physiol.*, 1901, **27**, 31.

and that the whole of the energy of the chemical changes is set free in the form of heat, we find that a resting gland weighing about 6 grammes produces about 1·1 calories per minute. We know, however, that a certain amount of external work is performed in the secretion of a saliva of lower osmotic pressure than the blood and also, when there is any resistance to the flow of saliva through the duct, in raising the hydrostatic pressure of the saliva in the duct.

Even during rest, changes are going on in the gland cells, changes leading to the formation of granules. When activity is excited by the stimulation of secretory nerves, the primary change appears to involve simply the granules. These absorb water, apparently against the osmotic pressure of the surrounding cytoplasm. Those nearest the lumen swell up, become converted into spheres containing water and salts in smaller proportion than exists in the fluid bathing the cells (and presumably in the protoplasm surrounding the granules), and in this swollen form are discharged or ruptured on the periphery of the cell into the lumen, so giving rise to secretion.

This discharge of a fluid with a smaller molecular concentration than the blood plasma must lead to an increased osmotic pressure in the remaining parts of the cell. This would induce a flow of water from tissue fluid into cell, and the consequent concentration of the tissue fluid would in the same way cause a flow of water into it from the blood. This pull of water by the cell from the blood is still further increased in another way. The act of secretion can be carried out only at the expense of chemical changes in the cell, which will result in the formation of a number of small molecules from the great colloid molecules of the protoplasm. The products of metabolism, or *metabolites*, will therefore accumulate in the cell, pass into the tissue fluid, and increase the osmotic pressure of the latter. This will call forth an increased transudation of fluid from the blood vessels, and will therefore produce a distension of the tissue spaces of the gland and an increased discharge of lymph along its efferent lymphatics.

As a secondary result of the activity there is a tendency to increased assimilatory changes and a preparation of the cell for further secretory changes, either immediately of hereafter. It was shown by Pavlov that the amount of nitrogen in the saliva formed by the submaxillary gland when the chorda was stimulated was greater than the amount of nitrogen lost by the gland itself. Anrep has shown that during the process of secretion the rebuilding of mucinogen either does not occur, or happens too slowly for detection, so that the mucin content of the saliva, on continuous chorda stimulation, steadily diminishes. The non-mucin nitrogen of the saliva is, however, not reduced on prolonged stimulation, and hence is being continually passed on from the blood. Replenishment of mucinogen after complete exhaustion may take as long as three days, and appears to be slightly accelerated by extirpation of the superior cervical ganglion. The submaxillary gland uses glucose from the blood stream at a rate which is accelerated when the gland secretes. (ANREP and CANNAN.*)

In the gland, as in muscle, when we attempt to form a conception of the mechanism of the chemical machine in the living cell, we are brought up against insuperable difficulties, and any theories so far put forward merely serve to show how far we are still from the actual comprehension of the events occurring in every living cell and underlying its conditions of rest and activity.

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2. MASTICATION AND DEGLUTITION

MASTICATION. In the act of mastication the food is mixed with saliva and ground by the teeth, aided by the cheeks, lips and tongue. Salivary secretion is augmented by mastication, and the mass of food, moistened and lubricated by the saliva, is finally rolled between the tongue and hard palate to form a bolus, preparatory to swallowing.

* ANREP and CANNAN. *J. Physiol.*, 1922, **56**, 248.

It has been shown by Sherrington * that mastication may consist of a series of reflexes, the closure of the jaws producing a stimulus to reflex opening; then, by rebound of the inhibited jaw-closure, another closure results, and so on, so long as anything biteable remains between the jaws.

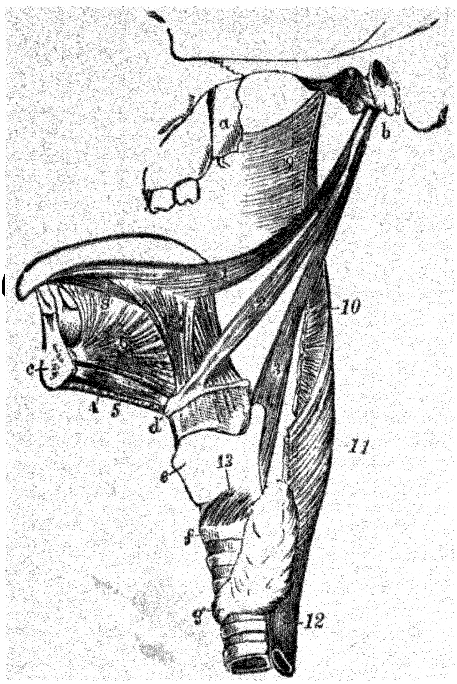

FIG. 535. Dissection to show Muscles Employed in Deglutition.

b, styloid process, from which arise 1, the stylo-glossus; 2, the stylo-hyoid; 3, the stylo-pharyngeal muscles; *c*, section of lower jaw, *d*, hyoid bone; *e*, thyroid cartilage; *g*, isthmus of thyroid gland; 4, cut edge of mylohyoid muscle; 5, 6, 7, 8, muscles of tongue; 9, 10, 11, superior, middle, and inferior constrictors of pharynx; 12, œsophagus. (ALLEN THOMSON.)

DEGLUTITION. The food after mastication is carried to the stomach by a complex series of co-ordinated movements involving the muscles of the mouth, pharynx and œsophagus (Fig. 535.)

Various methods have been used to study the process of deglutition in man and the higher animals. In various animals, *e.g.* ostrich, horse, giraffe, the movement of the bolus down the œsophagus can be seen or felt from the outside of the neck. Important information can be obtained by allowing a man or animal to swallow either a fluid or solid with which a bismuth salt or barium sulphate is mixed, and observing the passage of the opaque substance under the X-rays. The substance may be mixed with milk for a fluid, or with bread and milk for a semi-solid substance, or may be enclosed in a cachet and swallowed as a solid bolus. The various sounds produced in deglutition may be timed and suitably recorded in relation to other events. The time of entry of the food into the stomach may be determined by auscultating with a stethoscope over the region of the cardiac orifice. Since a certain amount of air is always swallowed at the same time as the food, the escape of this air through the small cardiac orifice gives rise to a bubbling noise which can be easily heard. The relative time relations of the events at different parts of the mouth, pharynx and œsophagus may be obtained by passing sounds either open, or provided with rubber balloons, to different levels, that for the pharynx being passed in through the nasal passage, and connecting these sounds with recording tambours or piston recorders. This method has been employed both in men and in animals, but the records from balloons are not easily interpreted, because a stationary balloon would probably be treated like a very large bolus, and be subjected to powerful efforts to move it onwards.

Observations of the movements of the soft palate and walls of the pharynx have been made also on patients suffering from wounds which exposed those structures to view.†

When a mouthful of water is taken, two sounds may be heard on auscultating over the œsophagus. The first sound immediately follows the beginning of the act of swallowing and is probably due to the impact of the fluid

* SHERRINGTON *J. Physiol.*, 1917, **51**, 420.

† COUVELAIRE and CROUZON, *J. de Physiol. et de Pathol. générale*, 1900, **2**, 280; PATEL and PELLANDE, *J. de Physiol. et de Pathol. générale*, 1903, **5**, 47.

against the posterior pharyngeal wall, brought about by the sudden contraction of the muscles which throw the fluid from the back of the tongue across the pharynx. The second sound is heard best by listening over the epigastrium. It begins from four to ten seconds after the first sound, and lasts for two or three seconds. The interval between the two sounds is not constant. If the observation be carried out on a man lying on his back, the trickling second sound is changed into a series of sounds which have been described as squirts, which vary from two to five in number, each lasting about one second. The second sound may be absent when a solid bolus is swallowed. On observing the process by X-rays, very much the same time relations are obtained. If a mouthful of milk mixed with barium sulphate be swallowed, it will be seen passing rapidly down the œsophagus to the cardiac orifice of the stomach. Here the passage is arrested for a time, and the fluid enters slowly in a narrow stream into the stomach. The average time which elapses between the beginning of deglutition and the disappearance of the last trace of fluid from the œsophagus is about six seconds (Fig. 536). A similar course of events is induced when the food swallowed is semi-solid. The bolus is shot at a surprising speed through the pharynx and upper œsophagus, but is slowed down at about the level of the clavicle, and travels more and more slowly till it reaches the cardiac orifice. If, however, the bolus be dry, such as a cachet, it may take as much as fifteen minutes to reach the cardiac orifice, although the individual who has swallowed it is quite unaware of its continued presence in the œsophagus. If, as would normally be the case, the cachet be well moistened with saliva or water before swallowing, it passes much more rapidly.

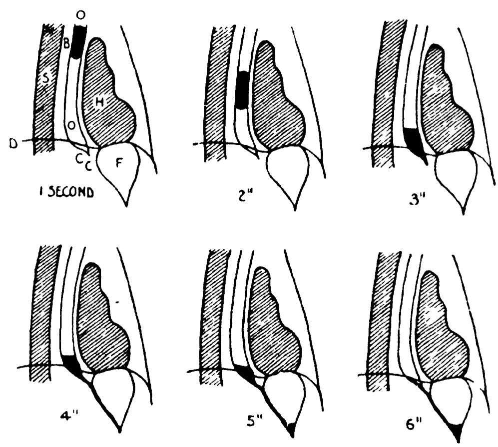

FIG. 536. Appearance of lower end of Œsophagus and Cardiac Sphincter, at one to six seconds after swallowing, observed under X-rays. (HURST.)

It has been customary since the time of Magendie to divide the act of swallowing into three stages: during the first, the bolus of food is carried past the anterior pillars of the fauces; during the second, through the pharynx, past the openings of the nasal cavities and of the larynx; and during the third, through the œsophagus into the stomach. There is, however, no pause between these various stages. The act of deglutition is one, and the initiation of the first stage inevitably involves the completion of the whole. The food when masticated is collected as a bolus on the dorsum of the tongue. A pause then takes place in the movements of mastication, and a slight movement of the diaphragm usually occurs known as 'respiration of swallowing.' An early event in swallowing is the closure of the mouth and nasal passages and slight raising of the larynx. If the thyroid cartilage is held down, or if the mouth is open to the pharynx, normal swallowing cannot occur. Next, a sudden elevation of the tongue throws the bolus back through the anterior pillars of the fauces. In this movement the chief factor is the contraction of the mylohyoid muscle, which presses the tongue

against the palate and pushes it backwards. The backward movement of the tongue may also be aided by the contraction of the styloglossus and palatoglossus muscles, which pull the base of the tongue suddenly backwards. These muscles, especially the palatoglossi, serve to close the isthmus faucium, thus preventing any return of the food towards the mouth.

As the food is passing through the upper part of the pharynx, it traverses a region common to the respiratory as well as the digestive passages. Its

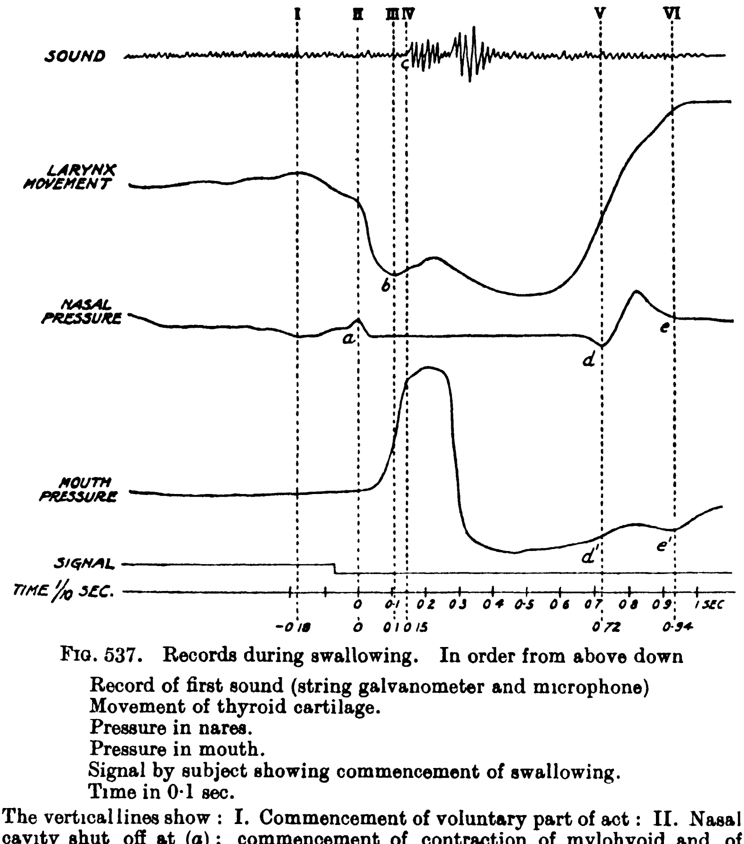

FIG. 537. Records during swallowing. In order from above down
Record of first sound (string galvanometer and microphone)
Movement of thyroid cartilage.
Pressure in nares.
Pressure in mouth.
Signal by subject showing commencement of swallowing.
Time in 0·1 sec.

The vertical lines show: I. Commencement of voluntary part of act: II. Nasal cavity shut off at (*a*); commencement of contraction of mylohyoid and of reflex part of act. III. Movement of larynx at (*b*); contraction of geniohyoid, genioglossus and hyoglossus. IV. Sound at (*c*). V. Opening of nasal cavity at (*d*), followed by rise of pressure. VI. End of act. (KAISER, *Arch. néerl de Physiol.*, 1918, I. 148.)

passage through this region is therefore rapid, and is associated with a closure of the two openings of the air passages into the pharynx. The nasal cavity is shut off by a simultaneous contraction of the levator palati and palato-pharyngeal muscles and azygos uvulæ, by which means the soft palate is raised (Fig. 538) and the posterior pillars are approximated to the uvula. The upper and back wall of the palate is thus formed into a tense sloping roof.

More important is the shutting off of the lower air passages. The contraction of the mylohyoid muscles, which initiates deglutition, is followed almost immediately (at an interval of 0·1 sec.) by an abrupt further elevation of the larynx, and this elevation is accompanied by closure of the glottis as

well as of the superior opening of the larynx. The laryngeal opening is bounded in front by the epiglottis, behind by the tips of the arytenoid cartilages, and at the sides, by the aryteno-epiglottidean folds. When deglutition takes place, the arytenoid cartilages, which normally lie against the posterior wall of the pharynx, are rotated and move inwards and forwards, so that the laryngeal opening assumes the form of a tri-radiate fissure, the vertical limb being short, while the transverse limb is rounded owing to the pulling inwards of the margins of the epiglottis. The epiglottis does not descend as a lid.* At the same time both the true and false vocal cords

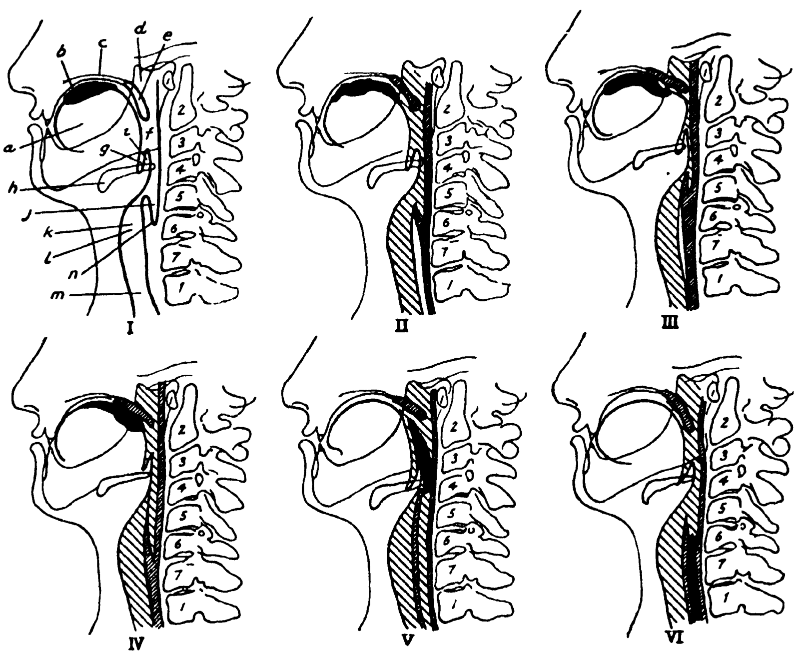

FIG. 538. Diagrams showing events in deglutition, as deduced from radioscopic observations. I. Before commencement: *a*, tongue, *b*, bolus, *c*, hard palate, *d*, nasopharynx, *e*, soft palate, *f*, pharynx, *g*, vallecula, *h*, hyoid, *i*, epiglottis, *j*, laryngeal pharynx, *m*, trachea, and *n*, œsophagus.

II. 1st stage, soft palate closing, larynx rising and pharynx narrowed.

III. 2nd stage: nose closed off, larynx raised, pharynx and upper larynx obliterated.

IV. Pharynx opening up, laryngeal pharynx drawn up to epiglottic position, œsophagus and upper larynx open.

V. Pharynx open, drawing bolus in.

VI. Laryngeal pharynx and œsophagus drop back with contents to normal position. (BARCLAY.)

come together, while the movement of the dorsum of the tongue backwards enables the closed laryngeal orifice to lie directly under the back part of the tongue. The muscles which are actively involved in this closure of the lower air passages are the external thyro-arytenoid, arytenoid, ary-epiglottidean, and the lateral crico-arytenoid muscles. Since the approximation of the posterior to the anterior boundary of the laryngeal opening is rendered possible only by the elevation of the whole larynx under the hyoid bone, the act of deglutition cannot be carried out unless the larynx is free to move.

The two openings from the back of the pharynx into the air passages

* ANDERSON STUART. *J. Physiol.*, 1906, 35, 446.

being thus closed, the bolus is shot rapidly past them into the region of the middle and inferior constrictors of the pharynx. If the bolus be liquid, the movement of the back part of the tongue may be sufficient to propel the substance past the constrictors through the lax œsophagus to its lower end. It is on this account that, when corrosive fluids are swallowed, we very often find the damage to the œsophagus limited to the three points where it is narrowed and there is a slight hindrance to the onward flow of fluid. If the bolus be large and semi-solid, it nevertheless passes through the pharynx with great speed ; one view of what happens here is that it is seized in the grasp of the middle constrictors on passing through the upper part of the pharynx, and is thrust by successive contractions of this muscle, and of the inferior constrictor, into the œsophagus. The walls of the cervical part of the œsophagus are composed of striated muscle. In the thorax, striated and unstriated muscles are associated together, while the lower third, in the neighbourhood of the stomach, consists almost entirely of unstriated muscle. Corresponding to these differences in structure, Kronecker and Meltzer * found differences in the duration and rapidity of propulsion of the contraction waves in each part. The contraction of the constrictors must be very rapid if this explanation is correct. Registration of pressures at different levels has not always given the same results in different investigations. According to Kronecker and Meltzer, a series of positive pressure changes travels along pharynx and œsophagus. Others have obtained negative pressures in the pharynx and upper œsophagus (*vide* Fig. 539) preceding the positive wave.† This would agree with the radioscopic observations of Barclay,‡ who describes the following events :

" 1. The mouth and nose are closed.

2. The larynx is raised and closed and its upper part is obliterated by the back of the tongue.

3. The pharyngeal space is obliterated by (*a*) raising the larynx, and (*b*) retraction of the tongue. (The posterior wall of the pharynx does not come forward ; the pharynx is not constricted but is compressed from before backward against the posterior wall, that is, against the vertebræ.)

4. The pharynx is opened up, and with the three outlets closed a negative pressure is obtained by (*a*) dropping the larynx, and (*b*) allowing the tongue to go forward.

5. During this movement the laryngeal pharynx still remains high, in close relation to the epiglottis. It is in an open-mouthed condition.

6. Food is tipped over the back of the tongue.

7. Food is sucked into the open mouth of the laryngeal pharynx, which, as it receives the bolus, drops from the epiglottis down to its normal position, thus opening up the larynx again, after the food has passed.

8. The food is probably sucked some distance down the œsophagus."

" The mechanism by which the larynx is cut off during the act is by a dragging up of the laryngeal pharynx behind the larynx to form contact with the epiglottis, possibly about a quarter of an inch below the tip of the epiglottis."

The free passage of liquids down the œsophagus under the influence of the propulsive force exercised by the mylohyoid muscles shows that the walls of this tube must be lax, and in fact one must assume that the first act of deglutition, so far as concerns the œsophagus, is an inhibition initiated

* KRONECKER and MELTZER. *Arch. f. Anat. u. Phys.*, 1880, 299. 446 ; 1883, *Suppl.* 328,

† SCHREIBER, *Arch. f. exp. Path. u. Pharm.*, 1901, **46**, 414 ; **67**, 72 ; DANIELOPOLU SIMICI and DIMITRIU, *J. de Physiol. et de Path.*, 1924, **22**, 595.

‡ BARCLAY. *Brit. Journ. Radiol.*, 1930, **3**, 534 ; *Acta Radiol.*, 1932, **13**, 91.

reflexly with the beginning of the act of deglutition. When a second act of deglutition succeeds the first with a sufficiently short interval, the reflex inhibition due to the second act may prevent the development of any wave of contraction in the œsophagus. This tube thus remains in a lax condition and allows the free rapid passage of the food downwards until the movements of deglutition have come to an end, when a peristaltic contraction occurs and sweeps all remaining adherent particles of food into the stomach.

The circular fibres of the lower end of the œsophagus, which form the cardiac sphincter of the stomach, are normally in a state of tonic contraction. When one mouthful of food is swallowed, it may either pass directly into the stomach by gravity, or remain at the lower end of the œsophagus until the following peristaltic wave forces it through the orifice. Entry into the stomach often occurs as an expiration begins. Distension of the œsophagus causes peristaltic œsophageal contractions (Fig. 539) of simpler form than those resulting from conscious swallowing; these contractions move the bolus forwards if it tends to stick. Considerable distension causes pain beneath the sternum.* When several acts of deglutition succeed one another, the cardiac sphincter shares in the inhibition of the œsophageal walls, and offers no resistance to the direct propulsion of food from the mouth to the stomach. Cannon has shown that the relaxation of the cardiac orifice which accompanies swallowing, extends also to the cardiac end of the stomach. This relaxation lowers the pressure within the stomach, and makes room for the incoming food.

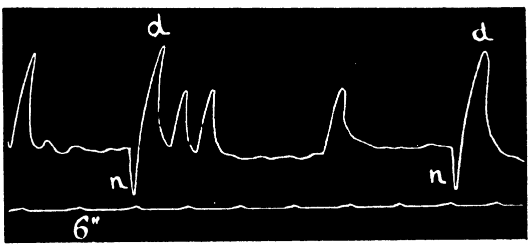

FIG. 539. Contractions of œsophagus recorded in man by a balloon method. At *n*, swallowing movements occurred. The subject was unaware of the other contractions. Note the negative wave as first event in conscious swallowing. (DANIELOPOLU *et al.*)

When the contents of the stomach are only feebly acid, the cardiac sphincter opens at intervals, especially if the pressure in the stomach is high, and allows of the regurgitation of the stomach contents into the lower part of the œsophagus. This is at once followed by a peristaltic contraction of this part of the œsophagus (apparently entirely unconscious), which drives the fluid back into the stomach. It has been supposed that movements of regurgitation become more and more infrequent as the gastric contents become acid; but the modern view associates the increasing tone of the sphincter with distension of the stomach and the stage of digestion.

The Nervous Mechanism of Deglutition. Deglutition is a reflex act. Swallowing reflexes are readily elicited in the decerebrate animal, when water or dilute alcohol is placed in the mouth. When we swallow voluntarily we supply the necessary initial stimulus, either by touching the fauces with the tongue, or by forcing saliva or food into the fauces. Normal swallowing is abolished by cocainisation of the mucosa of the pharynx. The afferent channels of the reflex are contained in the second division of the fifth nerve, the glosso-pharyngeal nerve, and the pharyngeal branches of the superior laryngeal nerve. We can excite a single act or a whole series of acts of deglutition by electrical stimulation of the central end of the last-named nerve. The efferent fibres travel by the hypoglossal nerve to the muscles of

* POULTON and PAYNE. *J. Physiol.*, 1922, **56**, Proc. liii.

the tongue, by the fifth to the mylohyoid, by the glossopharyngeal, the vagus and the spinal accessory nerves to the muscles of the fauces and pharynx. The closure of the larynx is effected by impulses which travel through the superior and inferior laryngeal branches of the vagus. Normal swallowing is impossible after section of both nerves near their origins. The centre for the act is situated in the medulla oblongata, and can be considered as consisting of a chain of centres, stimulation of one of which involves the firing off of all the others in orderly sequence ; the propulsion of the contraction down the œsophagus is determined by the intracentral nervous connections, and does not require the integrity of the muscular tube itself. If the œsophageal nerves be divided, the act of deglutition is abolished, the upper part of the œsophagus becoming permanently relaxed, while the lower part, including the cardiac sphincter, enters into a state of tonic contraction. On the other hand, the

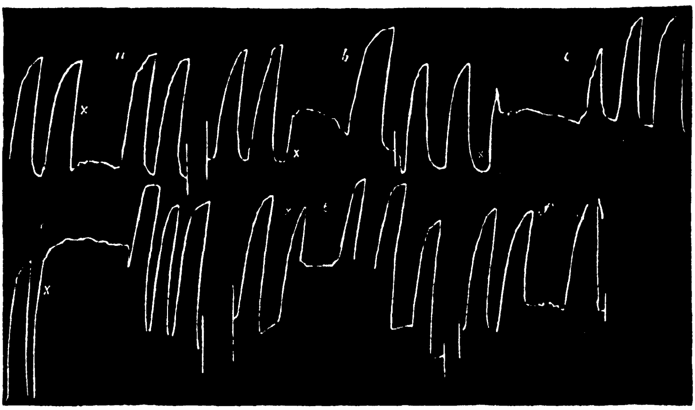

FIG. 540. Tracings of Respiratory Movements to show the Effect of Stimulating the Central End of the Glossopharyngeal Nerve. (MARCKWALD.)
The point of stimulation is marked with a cross. Note that the stoppage may occur at any phase of the respiratory movement.

œsophagus may be ligatured or cut across without interfering with the propulsion of the wave of contraction, started in the pharynx, from one end of the tube to the other. Stimulation applied to the mucous surface of the œsophageal tube is without effect.

There is an important interdependence between the functions of respiration and deglutition. If an inspiratory or expiratory movement were going on during the act of deglutition, food might be drawn into the lungs or driven into the nasal cavities. Such an accident is prevented by the fact that every act of swallowing inhibits a respiratory movement. This can be shown on oneself. If the breath be held until the feeling of dyspnœa becomes insistent, relief is at once experienced by swallowing, and the feeling of relief will last for three or four seconds. In some animals this inhibition appears to be effected reflexly through the glossopharyngeal nerve. Stimulation of the central end of this nerve in the rabbit * at once causes cessation of respiration in whatever phase it may happen to be (Fig. 540). This cessation lasts for five or six seconds, *i.e.* a sufficient length of time for a whole series of acts of deglutition. Respiration then recommences, and the inhibition cannot be prolonged by continuing the stimulation of the glossopharyngeal nerve. In other animals, *e.g.* cat, there

* MARCKWALD. *Z. f. Biol.*, 1887, **23**, 149, 239.

may be acceleration, while the dog gives variable results.* The afferent path is thus doubtful.

3. DIGESTION IN THE STOMACH

THE GASTRIC CONTENTS. The normal stomach several hours after food is rarely empty, but usually contains from 30 to 50 c.c. of fluid consisting of gastric juice, mucus, saliva and regurgitated duodenal contents. Within five minutes of the taking of food into the mouth, a secretion of gastric juice begins from the multitude of tubular glands which make up the greater part of the mucous membrane of the stomach. As the food is swallowed in successive portions, it accumulates in a mass in the body of the stomach, and the mass thus formed is penetrated with difficulty by the gastric juice,† so that salivary digestion can be continued for a considerable time. The flow of juice may continue for several hours and amounts in man, after a good meal, to 500 c.c. or more.

Gastric contents for examination can be obtained in various ways. The present method (fractional test meal) consists, after administering a standard meal (*e.g.* gruel), in withdrawing small samples of the contents at 15-minute intervals through a narrow tube which can be swallowed until its lower opening is about 20 inches from the incisor teeth, and left in place throughout the examination (Rehfuss).

GASTRIC JUICE. The earliest case in which human gastric secretion was adequately studied by direct observation was in a man in whom a permanent gastric fistula remained as the result of a gunshot wound, and who was kept under observation by Beaumont, an American Army surgeon, from 1825–33.‡

Human gastric juice can best be obtained by the subcutaneous injection of 0·5 mg. histamine hydrochloride (or 0·1 mg. per 10 kg.), the stomach having been previously washed out. In normal subjects this leads to a secretion of gastric juice at the rate of about 200 c.c. per hour: the juice as secreted probably contains 0·5 to 0·6 per cent. HCl, but this is reduced by neutralisation by mucus, which is also secreted throughout. The free acid of the juice varies considerably in consequence, the average in healthy men being about 0·39 per cent. HCl (= 107 c.c. n/10 per 100 c.c.; standard deviation 31·5; standard error 2·42) §; more individuals are above the mean than below it. The juice contains less pepsin in the later than in the earlier stages of secretion.||

It is sometimes necessary in a patient to perform gastrostomy, *i.e.* to make an artificial opening into the stomach through which he can be fed. In such patients it is observed that merely taking food into the mouth is followed by the secretion of gastric juice. Pavlov produced this condition artificially in dogs. The œsophagus was divided and the two ends brought to the surface of the neck. At the same time an opening was made into the stomach (Fig. 541). The animals could eat and swallow food, but the food thus swallowed fell out of the opening in the neck instead of passing into the stomach. Under these circumstances it is found that the "sham feeding" is quickly followed by a secretion of gastric juice. If taken from a fasting

* Teitelbaum and Ries. *Amer. J. Physiol.*, 1935, **112**, 684. (Various early workers also found acceleration.)

† Grützner. *Pflüger's Arch.*, 1905, **106**, 463.

‡ William Beaumont, "Experiments and Observations on the Gastric Juice and the Physiology of Digestion," Plattsburgh, 1833. (Reprinted on the occasion of the Thirteenth International Physiological Congress, Boston, 1929.)

§ Lee Lander and Maclagan. *Lancet*, 1934, **227**, 1210.

|| Helmer. *Amer. J. Physiol.*, 1934, **110**, 28.

animal, such a juice can be regarded as pure gastric juice. It is clear, colourless, strongly acid (pH about 1·0), and without smell. It contains no peptone, but traces of protein. The following table represents its average composition :

Hydrochloric acid	0·46 to 0·58 per cent.
Other Chloride	0·07 „ 0·5 „
Total solids	0·43 „ 0·65 „
Organic solids	— „ 0·45 „
Ash	0·09 „ 0·16 „
Total nitrogen	0·05 „ 0·08 „

Freezing point = − 0·55° C.

If the juice be allowed to stand at 0° C. for a day, it becomes cloudy and deposits a fine floccular precipitate, which contains most of the pepsin, the principal enzyme of the juice.

The actions of gastric juice are due partly to the acid, partly to the combined action of the acid and the enzymes. The acid of the gastric juice

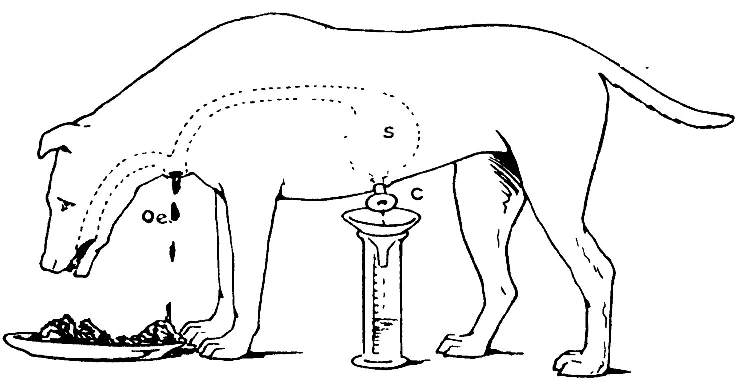

FIG. 541. Illustrating Pavlov's Method of obtaining Pure Gastric Juice Oe, the œsophagotomy opening ; S, the stomach, with fistula in which is fixed the silver cannula C

is entirely hydrochloric acid. Dog's juice contains on the average about 0·6 per cent. HCl. When, however, we examine the gastric contents, composed of a mixture of gastric juice and semi-digested food, we often find other acids besides the hydrochloric acid, and of these the most prominent is lactic acid. It is produced by fermentation occurring in the carbohydrates, converting them into sugar and then into lactic acid. As the gastric juice gradually soaks into the food and renders it acid, it stops this lactic acid fermentation.

Great stress has been laid on the determination of the actual amount of free H ions present, and for this purpose the acidity of gastric juice or of gastric contents can be measured by the use of suitable indicators, or more directly by the hydrogen electrode. The acidity estimated in this way is diminished considerably by the presence of proteins, proteoses or peptones, which act as weak buffers. But it does not seem that the adjuvant action of the acid on the proteolytic powers of the gastric enzyme is in any way affected by the diminution of its acidity ("combined HCl") caused by the presence of peptone.

In some pathological conditions, *e.g.* pernicious anæmia and gastric carcinoma, free hydrochloric acid may be entirely wanting from the gastric juice and the detection of this acid in gastric contents becomes therefore a matter of considerable clinical importance. For this purpose we can employ various indicators, which change colour in the presence of a strong acid such as HCl, but are unaffected by weak acids such as lactic acids or the

fatty acids. The reagent which is most employed is Gunzberg's reagent, a mixture of phloroglucinol and vanillin in absolute alcohol. A drop of this is evaporated to dryness. A drop of the fluid to be tested is then added, and also evaporated to dryness. If free HCl be present, the residue becomes a brilliant red colour, an effect which is not produced by the presence of free organic acids.

In order to determine quantitatively the amount of total acidity and free HCl, the gastric content is titrated with $N/10$ NaOH using thymol blue as indicator. The reading is taken when the red colour changes to yellow-orange, and gives the free HCl. The titration is then continued until the indicator turns blue; the further amount of alkali needed for this represents the combined HCl and organic and other weak acids. Probably the most accurate method is to titrate with standard NaOH after adding to the juice nine times its volume of acetone, using naphthylamine orange as indicator: end points are at pH 3·4 (pink) and pH 4·0 (orange). The weak acids are so much further weakened by the acetone that they scarcely require any alkali as compared with free HCl.* Estimation of total chlorides and mineral chlorides is also valuable.†

THE ACTIONS OF GASTRIC JUICE

Acid. By the action of the hydrochloric acid, certain changes are induced in the foodstuffs. Cane sugar is inverted to glucose and fructose; some proteins, such as blood fibrin, are swollen up to form a jelly-like mass. The caseinogen of milk is precipitated, the collagen of the connective tissues is swollen up. It is possible that a small amount of hydrolysis also takes place in the dextrins and maltose produced by the action of ptyalin on starch.

Pepsin. The chief digestive function of the gastric juice is dependent on the action of the enzyme, pepsin. Cathepsin is present also. It is an enzyme resembling pepsin, but differing from it in being active in neutral solution.

Pepsin, which is inactive in neutral medium, needs the co-operation of an acid (optimum of pH = 2·0), hydrochloric acid being the natural one. Its main effect is on the proteins of the food. The stages in its action may be best studied on blood fibrin. If fibrin be immersed in 0·4 per cent. hydrochloric acid, it swells up to a gelatinous mass. On adding an extract of gastric mucosa, or any preparation of pepsin, and warming to 37° C., the gelatinous mass rapidly undergoes solution. If the mixture be boiled and neutralised immediately after solution has occurred, nearly the whole of the protein is thrown down in a coagulated form. The first effect, therefore, is the production of soluble heat-coagulable proteins from the insoluble fibrin. If the action be allowed to proceed for some hours, a whole series of products of hydrolysis is found in the mixture. On neutralising the fluid, a precipitate may be thrown down consisting chiefly of *acid meta-protein.* The greater proportion of the protein remains in solution. After boiling and filtering, the filtrate contains *proteoses* and *peptones.* The proteoses are precipitated by saturation with ammonium sulphate, by which peptones are not precipitated. In the course of peptic digestion it can be shown that COOH groups and NH_2 groups become liberated, and in equal numbers,‡ which shows that pepsin acts by breaking certain of the peptide linkages of proteins, thus :—

$$\cdots R.CO.NH.R'\cdots + H_2O = \cdots R.COOH + NH_2.R'\cdots$$

After completion of its action, about one-tenth of the total peptide linkages of the protein molecule have been severed in this way.

Proceeding from primary through secondary proteoses to peptones, there

* Richardson. *Proc. Roy. Soc.*, 1934, **115B**, 170.

† *v.* Harrison. "Chemical Methods in Clinical Medicine," 1930. (Churchill.)

‡ Waldschmidt-Leitz and Künstner. *Z. f. Phys. Chem.*, 1927, **171**, 70.

is a continuous diminution in the size of the molecule. During the time over which gastric juice can exert its influence, a maximum, say, of eight hours, the breakdown never passes beyond the proteose and peptone stage, and it is in this form that the proteins of the food pass on into the small intestine.

Action on particular Proteins. *Collagen.* The connective tissues, *e.g.* areolar tissue, tendon and bone, are made up chiefly of collagen. On prolonged boiling it is converted into gelatin. The gastric juice dissolves collagen, converting it, probably through the stage of gelatin, into gelatoses and gelatin peptones, bearing the same relation to the original substance as is borne by the proteoses and peptones to the proteins. On account of this action, adipose tissue is broken up. The cellular envelopes are dissolved, and the fat floats freely in the gastric juice.

Elastin, the chief constituent of elastic fibres, may be regarded as indigestible.

Mucin, which forms a considerable proportion of the ground substance of connective tissues, is converted by gastric juice into peptone-like substances, and into reducing bodies allied to glucosamine.

The *Nucleo-proteins* are first dissolved by the acid of the gastric juice, and are then broken up into two moieties. The protein half is converted into proteoses and peptones, while the nuclein moiety is precipitated, as *nucleic acid.*

Phospho-proteins undergo special changes in the stomach. Caseinogen of milk is present in the form of calcium *caseinogenate.** The first effect of gastric juice, even in neutral medium, is to convert the caseinogen into an insoluble form called *casein.* This action is due to the presence of a distinct enzyme of the gastric juice, named *rennin.* Although crystalline pepsin also clots milk, an active rennin preparation can be obtained which has no peptic activity, is a thioproteose and in various other respects differs chemically from pepsin.† For the conversion of caseinogen into the solid clot of casein the presence of calcium salts is necessary. The addition of rennin to oxalated milk apparently produces no effect, but clotting ensues if calcium chloride is then added to the mixture. It is therefore thought that, in the clotting of milk, the caseinogen under the action of the rennin first undergoes conversion into casein, the sodium salt of which is soluble, but the calcium salt insoluble. Hence if calcium ions are present, calcium caseinate, or insoluble casein, is precipitated and causes the solidification of the milk. Under the further action of the acid gastric juice, the clot of casein first formed is dissolved, but a precipitate is left containing a small proportion of the original phosphorus of the caseinogen. This precipitate is sometimes spoken of as para-nuclein, or *pseudo-nuclein.* It does not yield purine bases on hydrolysis with acids, as nucleic acid does, but contains phosphoric acid in organic combination. By prolonged digestion it is possible to dissolve the whole of the precipitate.

Action on Carbohydrates. The chief digestion of carbohydrate which takes place in the stomach is that of starch, since the amylase of the swallowed saliva continues to act until the contents become definitely acid. It seems probable that the hydrolysis of cane sugar and some other carbohydrates, *e.g.* inulin, which takes place in the stomach can be completely accounted for by the action of hydrochloric acid present, and that there is no need to assume the presence of a special enzyme.

* In the U.S.A. and on the Continent caseinogen and casein are respectively called *casein* and *paracasein.*

† TAUBER and KLEINER. *J. Biol. Chem.*, 1932, **96**, 745 ; 1934, **104**, 259

Action on Fats. The chief action of this juice on fats is the solution of their connective tissue framework and protoplasmic envelopes, so as to set the fat free in the gastric contents. After a fatty meal it is found, moreover, that a proportion of the fat in the stomach has undergone hydrolysis with liberation of free fatty acid. In this hydrolysis three factors are involved: (1) the action of the warm dilute hydrochloric acid; (2) the action of a *lipase*, which is secreted by the walls of the stomach, and acts especially at the beginning of gastric digestion before the contents have attained a high degree of acidity. The action of this enzyme is conspicuous only if the fat be present in a finely divided form, as in yolk of egg; (3) the action of lipase regurgitated from the duodenum. In any case, the chief digestion of fat takes place in the duodenum.

ANTI-ANÆMIC FACTOR. Gastric juice also contains an important principle, called the *intrinsic factor* which reacts with substances present in certain foods to form a substance essential to the proper formation of red blood corpuscles. The intrinsic factor is thermolabile and may be an enzyme, but if so is not pepsin or rennin.

THE SECRETION OF GASTRIC JUICE

The stomach consists of the *cardiac portion*, the *corpus* or main body, and the *pyloric part*. There are definite differences between these as regards their functions.

Histological investigations show that four types of cells are recognisable in the gastric mucosa, viz. surface, mucoid, peptic and oxyntic cells (Lim).* The surface cells cover the inner surface of the mucosa, and line the ducts of the tubular gastric glands; they secrete mucus. Mucoid cells form the secreting cells of those pyloric and cardiac glands which are placed near the two sphincters; they are also mingled with the peptic cells lining the cardiac glands. They are probably closely connected in function with the peptic cells, since in new-born animals they precede these in the cardiac glands.

Oxyntic cells are absent from the pyloric part of the mucosa, and the juice secreted in the pyloric portion contains no free HCl. A survey of the distribution of enzymes in sections of gastric mucosa, when correlated with the distribution of the various cells shows that HCl is related to oxyntic cells, pepsin to peptic cells and a peptidase to the peptic and neck cells.†

The pepsin is not present as such in the resting peptic cells, but is represented by a zymogen, *pepsinogen*, present in the form of granules.‡ As secretion proceeds the granules diminish in number, and are confined to the inner margin of the cell. Pepsinogen has been isolated in pure crystalline form from the mucosa of the body of the stomach.§ It is converted into pepsin in solutions more acid than pH.6, and in the process about 15 per cent. of the nitrogen content is split off.

Histological changes also occur in the oxyntic cells when secretion occurs.‖ The formation of free hydrochloric acid by the oxyntic cells is probably the result of an interaction between carbonic acid, chlorides and phosphates. It has been shown by Collip that during the resting state these cells contain abundance of phosphate, but little or no chloride, whereas both salts are present in the peptic cells and in the connective tissue. During activity, chlorides are found in abundance in the oxyntic cells. It would seem probable therefore that, perhaps owing to increased production of CO_2, hydrochloric acid is formed in the oxyntic cells, much as happens in blood when CO_2 is added to it and, by the Hamburger ionic interchange (p. 770), HCl is set free in the cells, and

* LIM. *Q. J. Micr. Sci.*, 1922, **66**, 187.
† LINDERSTROM-LANG *et al.* *C. R. Lab. Carlsberg*, 1935, **20**, 1, 66.
‡ LANGLEY. *J. Physiol.*, 1882, **3**, 269.
§ HERRIOTT. *J. Gen. Physiol.*, 1938, **21**, 501.
‖ CARLIER. *Proc. R. S. Edin.*, 1899, **22**, 673.

$NaHCO_3$ increased in the plasma. No doubt carbonic anhydrase plays a part in accelerating the ionisation of H_2CO_3. There is an even higher concentration of carbonic anhydrase in the oxyntic cells than there is in red blood corpuscles; in the peptic cells there is very little.*

There certainly appears to be a close relationship between the total CO_2 content of the blood and the volume or acidity of the gastric juice secreted † under a constant stimulus, and it has also been claimed that the amount of acid secreted is increased by a rise and diminished by a fall of the free CO_2 of the blood.‡ When dyes are injected into the blood, only those with an electropositive coloured ion are secreted into the gastric juice, those with electronegative chromogen ions being retained in the blood. It is suggested that this is due to an electric charge on the secreting membrane, which allows chlorine ions to pass out in exchange for carbonate or some other anion, which passes in.§ If this membrane were the oxyntic cell, this might pass on the HCl into the lumen of the tubule as fast as it is formed, and the bicarbonate the opposite way into the lymph, so that the reaction would be continuous.

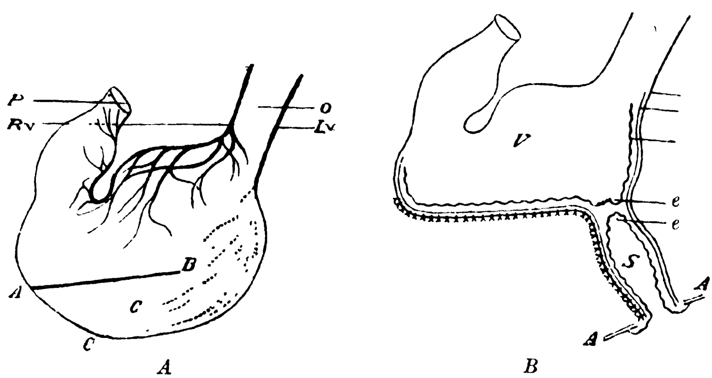

FIG. 542. Diagram to show Pavlov's Method of making a Cul-de-sac of the Body of the Stomach, with Vascular and Nerve Supply intact.

In A the line of the incision into the gastric wall is shown. B represents the operation as completed.

In A: *O*, œsophagus: *R v*, *L.v.*, right and left vagus nerves; *P*, pylorus; *C*, corpus, or body of stomach; *A.B.* line of incision.

In B: *V*, main portion of stomach; *S*, the cul-de-sac; *A*, abdominal wall; *e*, *e*, mucous membrane reflected to form diaphragm between the two cavities.

The normal resting stomach secretes some gastric juice, which appears to consist of a mucilaginous secretion, often rich in pepsin, but containing little or no acid.

Reflex Secretion In the 'sham feeding' experiment carried out as already described, secretion of gastric juice rich in acid begins in about five minutes after the onset of feeding, and continues as long as this goes on, which may be for several hours. This method does not, however, enable us to determine whether the character of the juice will be altered in any way by the changes which the food undergoes in the stomach itself. In order to form an idea of the normal course of secretion of gastric juice when food is taken into the stomach in the ordinary way, Pavlov devised another procedure. A small diverticulum, representing about one-tenth to one-fifth of the whole stomach, is made, usually in the body of the stomach, in direct muscular and nervous continuity with the rest of the stomach, but shut off from the main part of the viscus by a double diaphragm of mucous membrane (Figs. 542, 543). In a dog treated in this way it is found

* DAVENPORT. *J. Physiol.*, 1939, **97**, 32.

† APPERLY and CRABTREE, *J. Physiol.* 1931, **73**, 331; BROWNE and VINEBERG, *ibid.*, 1932, **75**, 345.

‡ THEORELL. *Skand. Arch. f. Physiol.*, 1933, **66**, 225.

§ INGRAHAM and VISSCHER. *J. Gen. Physiol.* ,1935, **18**, 695.

that the amount of juice secreted by the small stomach bears always the same ratio to the amount secreted by the large stomach, while the digestive power of the juice obtained from the small stomach is equal to that obtained from the large. This is shown in the following Table, from an experiment in which

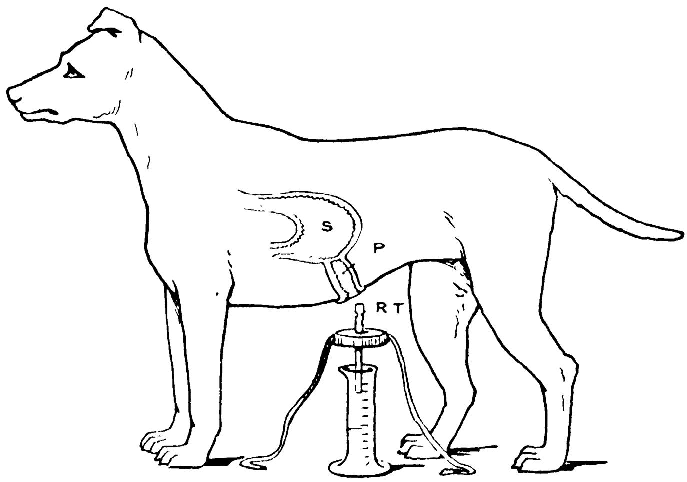

FIG 543 Illustrating the Principle of the Miniature Stomach, or "Pavlov Pouch." S, body of stomach, P, the miniature stomach, R T, cannula made of india-rubber tubing, which is inserted into P, and held in position by tapes when juice is to be collected.

there was also a fistula into the main stomach, and in which secretion was excited by sham feeding:

SECRETION FROM GASTRIC FISTULÆ AFTER SHAM MEAL

Hours.	Small stomach.		Large stomach	
	Quantity	Strength *	Quantity.	Strength
1	7·6 c.c.	5·88 mm.	68·25 c.c.	5·5 mm.
2	4·7 c.c.	5·75 mm.	41·5 c.c.	5·5 mm.
3	1·1 c.c.	5·5 mm.	14 0 c.c.	5 38 mm.
Total . .	13·4 c.c.	—	123·75 c.c.	—

We may therefore regard the secretion obtained from the small stomach as a sample of that produced by the large, and from the changes in this small stomach either study the effects of a normal meal in which the food is swallowed, or of a sham meal in which the food is merely masticated, or of a meal directly introduced into an opening into the large stomach.

The fact that sham feeding causes the production of gastric juice shows that we have to do, in the first place, with a reflex nervous mechanism. This is an unconditioned reflex. But a secretion, which is at least as vigorous as that produced by a sham meal, can be evoked through a conditioned reflex, by merely providing some stimulus associated with the animal's food habits. If the animal be hungry, it is sufficient to show it the food to produce this effect. In the experiment from which the following Table is taken, the dog

*** The strength of the juice was determined by measuring the number of millimetres of coagulated egg-white (in Mett's tubes) which were digested in eight hours.**

was continually excited by showing it meat during a period of an hour and a half. At the end of this time the animal, which had an œsophageal fistula, was given a sham meal. It will be observed that the 'psychical secretion' obtained during the first period of the experiment was rather greater than the secretion produced by the introduction of food into the mouth.

PSYCHICAL SECRETION OF GASTRIC JUICE (PAVLOV)

Period.	Quantity.
8 minutes	10 c.c.
4 "	10 "
4 "	10 "
10 "	10 "
10 "	10 "
8 "	10 "
8 "	10 "
19 "	10 "
9 "	3 "

SHAM FEEDING

Period.	Quantity.
17 minutes	10 c.c.
9 "	10 "
8 "	10 "

The afferent channels for this reflex may be either the afferent nerves from the mouth or, in the conditioned reflex, any of the nerves of special sense, such as sight, which have, by being brought into use in association with the unconditioned reflex, acquired the properties of conditioned afferents.

The efferent channels can be only one of two nerves, the vagus and the sympathetic. That the former of these nerves is involved is shown by the fact, recorded by Pavlov, that psychical secretion, as well as the results of a sham meal, is entirely abolished by division of both vagi.

The converse experiment of exciting secretion by direct stimulation of the vagus has been carried out by Pavlov as follows: An animal with fistulæ of œsophagus and stomach had one vagus nerve divided in the neck. A thread was attached to the peripheral end of the cut vagus and allowed to hang out through the wound. Four days after the operation, the vagus was drawn out of the wound and its peripheral end stimulated. No effect was produced on the heart, owing to the degeneration of the cardio-inhibitory fibres. Five minutes after the commencement of the stimulation the first drop of gastric juice appeared from the gastric cannula, and a steady secretion of juice was obtained with continuation of the stimulation. A striking fact is the great length of the latent period before gastric secretion begins. This has not yet been satisfactorily explained. The vagus also controls secretion of all the mucous and mucoid cells of the stomach.* It also contains secretory inhibitory fibres, which have the effect of stopping or diminishing the secretion under certain conditions.

The sympathetic supply is vaso-constrictor, but stimulation of the splanchnic nerves five days after section (to allow degeneration of vaso-constrictors) gives a secretion of gastric juice, as also, according to most observers, does the injection of adrenaline. In emotional states the secretion of gastric juice is greatly reduced.

Chemical (Humoral) Secretion. These experiments show conclusively that an important—probably the most important—part of the gastric secretion is determined by a nervous mechanism. This nervous secretion does not, however, account for the whole of the gastric juice obtained as the result of a meal, since further secretion is effected in consequence of the presence of certain substances in the stomach itself, or in the duodenum. If a vagotomised animal is provided with two gastric fistulæ, one into a diverticulum and the other into the main stomach, it is found that the introduction of meat into the large stomach is followed, after a period of

* JENNINGS and FLOREY. *Q. J. Exp. Physiol.*, 1941, **30**, 329.

twenty to forty-five minutes, by a secretion of gastric juice from the small stomach. Moreover, when an animal is given a normal meal and swallows the food normally after mastication, the total amount of gastric juice obtained is greater, and the flow is of longer duration, than that produced by the sham feeding alone. This combined character of the gastric juice produced by a normal meal is shown in the following table (Pavlov):

SECRETION OF GASTRIC JUICE

Hours.	Normal meal. 200 grm. meat into stomach.		150 grm. meat direct into stomach.		Sham meal.		Sum of last two experiments.
	Quantity c c	Strength mm.	Quantity c.c	Strength mm.	Quantity c c.	Strength mm	Quantity c c.
1	12·4	5·43	5·0	2·5	7·7	6·4	12·7
2	13·5	3·63	7·8	2·75	4·5	5·3	12·3
3	7 5	3·5	6·4	3·75	0 6	5·75	7·0
4	4·2	3·12	5·0	3·75	0	0	5·0

In the first section is given the result of a normal meal on the secretion from the gastric diverticulum. In the second section are given the amount and digestive power of the juice which is excited by the direct introduction of 150 grammes of meat into the large stomach of the animal, care being taken not to excite the reflex mechanism. In the third section are given the amount and digestive power of the juice which is evoked by a sham meal of 200 grammes of meat. In the fourth section is given the sum of the last two experiments. It will be seen that the total effect of the sham meal *plus* the direct introduction of meat into the stomach is almost identical with the secretion obtained when the food is taken in a normal way.

Edkins * showed that a chemical mechanism may account for the second phase of the secretion of gastric juice. The lower end of the œsophagus was tied off so as to occlude the cardiac orifice and crush the two vagus nerves. Warm normal salt solution was introduced into the stomach and kept there at a constant pressure. Control observations showed that the mere introduction of this fluid into the stomach caused no secretion of gastric juice. The injection of peptone, of acid, of broth or of dextrin into the blood stream also produced no secretion. If, however, in the course of the hour during which the fluid was allowed to remain in the stomach, a decoction made by boiling pyloric mucous membrane with acid, with water or with peptones, was introduced in small quantities every ten minutes into the jugular vein, the fluid removed at the end of the hour was found to be distinctly acid in its reaction and to possess proteolytic properties. The injection of these substances had therefore caused the secretion of a certain amount of gastric juice. Edkins concluded therefore that the first products of digestion act on the pyloric mucous membrane, and extract from this membrane a substance which is absorbed into the blood stream, and carried to the glands of the stomach, where it acts as a specific excitant of their secretory activity. This substance was called *gastrin* or the *gastric hormone*.

This second phase of secretion certainly cannot be ascribed to the intervention of any reflex, since it occurs after cutting off the stomach from its connections with the central nervous system. Ivy and Farrell † have trans-

* EDKINS. *J. Physiol.*, 1906, **34**, 133.
† IVY and FARRELL. *Amer. J. Physiol.*, 1925, **74**, 639.

planted a denervated miniature stomach into the mammary region, and found that the second phase still occurred in it about three to four hours after the animal was fed. Its cause must be something carried by the blood.* It cannot be due to mechanical stimulation, since the pouch is empty. Moreover it is not produced by all sorts of food. The introduction of white of egg, of starch or of bread into the stomach causes no secretion. On the other hand, if bread and gastric juice be allowed to digest for some time, the introduction of the mixture into the stomach evokes a secretion. So also will glucose, alcohol, lactic acid, sodium bicarbonate, soap, saliva, or meat; still more potent than meat, however, is a decoction of meat, or bouillon, Liebig's extract of meat, peptone or albumoses. The exciting mechanism must be some chemical substances present in meat, and produced in various other foods under the action of the first gastric juice secreted in response to nervous stimuli. Certain substances, *e.g.* pilocarpine and histamine, are known to produce gastric secretion when injected into the blood stream, but though the juice formed contains the normal amount of acid, it is poor in solids and in pepsin.†

After resection of the pyloric part of the stomach this second phase is absent (Smidt) The chemical theory of the second phase of gastric secretion has been fully confirmed by Savitsch and others. Savitsch carried out the surgical operation of isolating the pylorus from corpus and duodenum, dividing all its nerves, and making fistulæ into this isolated pylorus and into the body. Introduction of chemical excitants into the pylorus evoked gastric secretion in the corpus. It has been suggested that histamine is identical with the gastric hormone. This is rendered unlikely by the fact that the action of the chemical excitants of gastric secretion, but not that of histamine, is inhibited by atropine. Moreover, pyloric extracts free from histamine excite gastric secretion when injected intravenously,‡ whether atropine has been given or not. It is probable that histamine is liberated in the cells by the action, either of acetyl choline from vagus stimulation (abolished by atropine), or by the presence of gastrin §

Pavlov showed that the second phase of the gastric secretion is largely influenced by the character of the contents of the stomach. Thus after the ingestion of large quantities of fat, the secretion of gastric juice during the second phase is greatly delayed or inhibited and also reduced in amount, and in acidity and pepsin content. After some of the fatty contents have been passed into the duodenum, there is considerable regurgitation of emulsified fat and duodenal contents into the stomach, so that the volume of the gastric contents is subsequently increased. The inhibitory effect of the fat seems to be exerted from the duodenum and not from the stomach itself, as may be shown by the results of introducing fat into stomach or duodenum when these have been separated from one another. After the preliminary delay, gastric secretion commences, often three or four hours late, and may then be quite brisk. This secretion is due to the action of the soaps which have been formed in the meantime by digestion of the fat by the regurgitated duodenal contents, upon the pyloric portion of the stomach. The main conclusions on the action of fat have been confirmed on man, *e.g.* when oil is introduced directly into the duodenum, or given before the meal, the inhibition of gastric secretion is greater than when it is swallowed with or after a meal.||

The Normal Course of Secretion. We may say that the gastric secretion in response to a normal meal shows three phases: (1) a *reflex phase*,

* LIM, LOO and LIU. *Chinese J. Physiol.*, 1927 **1**, 51.
† BABKIN. *Canad. Med. Assoc. Journ.*, 1930, **23**, 268.
‡ KOMAROV. *Amer. J. Physiol.*, 1938, **123**, 121.
§ BABKIN. *Can. Med. Assoc. J*, 1938, **38**, 421.
|| ROBERTS. *Quart. J. Med.*, 1931, **24**, 133.

which begins within five minutes of the taking of the food and is determined by the reflex nervous mechanism described above ; and (2) a smaller portion, the *second phase,* due to gastrin, and excited by the presence of the food in the stomach ; (3) a *third phase,* small and variable, and due to the presence of food in the duodenum. The normal gastric secretion is mainly due to the co-operation of the first two factors. The first and most important is the reflex secretion, determined through the vagus nerves by stimulation of the mucous membrane of the mouth, or by conditioned reflexes. The second factor, which provides for the continued secretion of gastric juice long after the reflex effects of feeding have disappeared, depends on the extraction from the pyloric mucous membrane of gastrin, which acts as a chemical messenger to all parts of the stomach, being absorbed into the blood and thence exciting the activity of the various secreting cells in the gastric glands.

THE THIRD, OR INTESTINAL PHASE of gastric secretion, is similarly due to the formation, in the duodenum or upper part of the small intestine, of a gastric hormone which

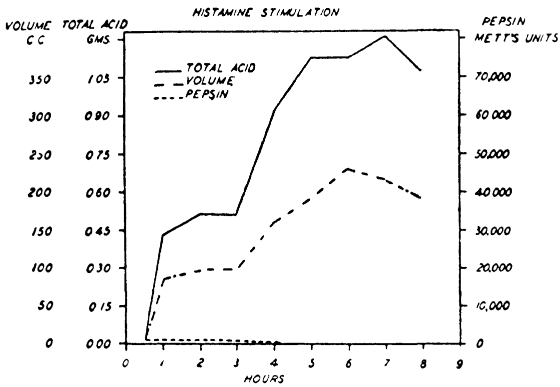

FIG 544 Gastric juice resulting from hourly injections of histamine. The pepsin falls to zero, while acid and volume increase. (BOWIE and VINEBERG *Q. J. Exp. Physiol.*, 1935, **25**, 247.)

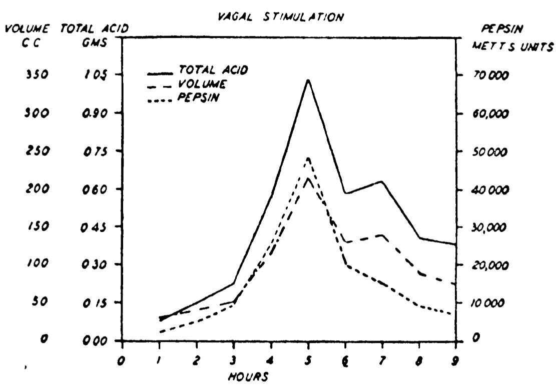

FIG. 545. Gastric juice from nine hours' vagus stimulation. Pepsin and acid are maintained. (BOWIE and VINEBERG.)

enters the circulating blood. It is relatively much smaller in amount than the second phase, and is demonstrated on dogs with gastric and duodenal fistulæ and with the stomach closed off from the duodenum, or else by uniting the œsophagus with the

duodenum so as to isolate the stomach altogether (Ivy, Lim and McCarthy*). Gastric secretion results when various substances are introduced into the duodenum, *e.g.* water, meat, meat extract, peptones and other products of protein digestion, &c., while *inhibition* of secretion is said to occur when sodium bicarbonate is introduced. With the sole exception of dilute alcohol, introduction of no substance into the large intestine produces any secretion.

As sodium bicarbonate is so often administered to patients, its effects on gastric secretion may be noted. Taken on an empty stomach it passes direct to the duodenum

A. B.

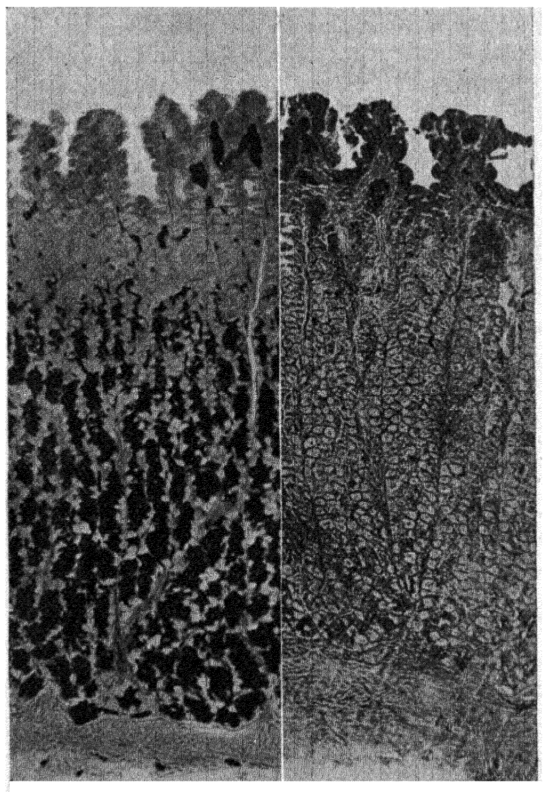

FIG. 546. A. Histological appearance of gastric mucosa after eight hours of histamine injection. The pepsinogen content is normal. B. Histological appearance of gastric mucosa after nine hours' vagal stimulation. The pepsinogen granules have nearly all vanished. (BOWIE and VINEBERG.)

and inhibits gastric secretion. Taken with or after food it increases secretion by its effect on the pyloric mucosa.

A further important question is whether there is any alteration in the composition and amount of gastric juice with variations in the character of the food. So far as concerns the first phrase of secretion, the 'appetite' juice, Pavlov showed that, whatever the previous diet, the juice always has the same characters. But in the case of the second secretion, there is considerable variation in the nature of the juice. Whereas the secretion of juice is greatest in amount after a meal of meat, the digestive power of the juice is greatest after one of bread. We have seen that the psychical juice depends merely on appetite, and therefore will be greater in amount the more welcome the food. On the other hand, the juice secreted in the second phase must vary according

* IVY, LIM and MCCARTHY. *Amer. J. Physiol.*, 1925, 74, 616.

to the quantity of gastric hormone produced, and therefore with the nature and amount of the substances produced in the preliminary digestion of the gastric contents by means of the psychic juice. The amount of juice may vary also with the salts contained in the food, according to their alkaline or acid character; and the percentage of pepsin in the juice may vary with the intensity of stimulus as well as with the quantity of fluid available for the formation of the gastric juice. Fats reduce the gastric secretion and prolong the stay in the stomach. Water, tea or coffee taken with food augment gastric secretion, and various articles of food, such as vegetable juices, contain powerful chemical excitants (*e.g.* histamine in spinach). Again, slight distension of the pylorus, though not capable of producing secretion, exerts an augmenting effect when gastric secretion is in progress. These factors will co-operate in determining the characters of the whole juice secreted after any given meal.

According to Babkin * the vagus or reflex secretion is responsible for the control of the mucus, acid and pepsin, perhaps separately from one another, while the water and acid are mainly affected by the humoral control. These views have received strong confirmation from the work of Bowie and Vineberg.† They showed that when gastric secretion was excited for several hours by repeated injections of histamine, the pepsin content of the juice soon fell to zero, while the acid secretion continued (Fig. 544). Prolonged vagal stimulation, however, gave a juice rich in both acid and pepsin (Fig. 545), and vagus stimulation also caused the appearance of pepsin in the juice from which it had vanished after induction of secretion by histamine. When the gastric glands were specially stained to show the presence of pepsinogen, this was found to be still abundant, as in the resting stomach, after prolonged secretion by histamine, whereas after prolonged vagal secretion the pepsinogen granules had almost entirely disappeared (Figs. 546 A and B). Babkin supposes that the varying characters of the gastric juice are due to different amounts of activity in the four types of cells which may secrete in the gastric glands.

The acidity of gastric juice is constant, or nearly so, at about 0·135 N or 0·5 p.c. HCl, during the height of secretion. At the beginning and end, the juice is mixed with mucus, which dilutes and neutralises the acid.‡ Irritation of the mucosa by chemical or mechanical means increases the amount of mucus secreted.

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4. THE MOVEMENTS OF THE STOMACH

These can be best studied by observation by means of the X-rays. In order to make the shape of the stomach visible, the food—bread and milk—is mixed with a quantity of barium sulphate. The presence of this does not interfere with the processes of digestion, but renders the gastric contents opaque to the Röntgen rays.

In the human stomach the term *fundus* is limited to that part of the stomach situated above the cardiac orifice (in the erect position). The *body* of the stomach is marked off from the pyloric part by the *incisura angularis* on the lesser curvature, represented in many animals by a strong "transverse band" (Fig. 547.) The pyloric portion consists of the *pyloric vestibule* (or *antrum*) and the *pyloric canal*, the latter being a tubular portion with thick muscular walls about 3·0 cm. in length. A somewhat thicker ring of plain muscle surrounds the canal at its junction with the duodenum, and is called the *pyloric sphincter*.

* Babkin. *Canad. Med. Assoc. Journ.*, 1931, **25**, 134.

† Bowie and Vineberg. *Q. J. Exp. Physi.*, 1935, **25**, 247.

‡ Bolton and Goodhart. *J. Physiol.*, 1931, **73**, 115; Helmer, *Amer. J. Physiol.*, 1934, **110**, 28.

When food has been swallowed (in the erect position) its weight is sufficient to overcome the resistance of the contracted gastric wall and some of it rapidly passes to the pyloric part. The remainder stays in the body of the stomach, which keeps constant pressure on its contents, tending to force them towards the pylorus. Contraction waves begin almost at once, each constriction starting near the middle of the stomach, and deepening as it slowly progresses towards the pylorus (Figs. 547, 548). These waves succeed one another, so that the pyloric part may present a series of constrictions. Their effect is to force towards the pylorus the food which has been mixed with gastric juice and detached from the surface of the mass in the corpus. If the pylorus remains closed the food cannot escape, and therefore is squeezed back, forming an axial reflux stream towards the body. It may be doubted, however, whether the pyloric sphincter remains as persistently contracted as was formerly believed *; in fact it has been claimed that it is normally relaxed, closing only after a wave has passed over it from pyloric antrum to duodenum, this closure preventing reflux from the duodenum.† The pyloric contractions last throughout the whole period of gastric digestion, and become more marked as it proceeds. By their action a thorough mixture of food and gastric juice results. Each wave as it travels along exhibits every two to three seconds the rhythmic fluctuations in intensity common to most plain muscle contractions, and when a wave at a phase of increase reaches a point about one inch from the pyloric canal it is so marked that part of the pyloric vestible becomes almost completely separated from the rest of the stomach. The part thus cut off then diminishes in size in every direction,‡ part of its contents being forced through the pyloric canal, while the remainder escapes back as an axial reflux stream into the stomach. The waves recur at regular intervals of fifteen to twenty seconds, and three or four are present simultaneously. They continue without cessation until the stomach is empty—from one to four hours after the meal, according to its bulk and composition.

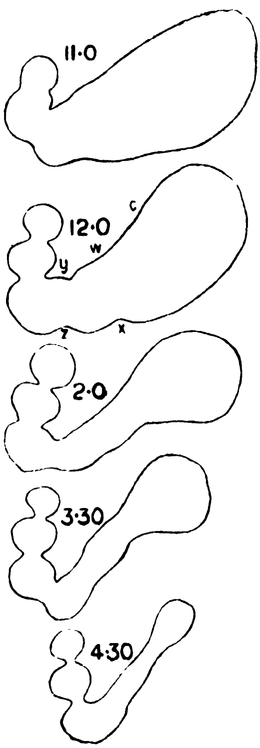

FIG. 547. Shadow Sketches of the Outlines of the Stomach of a Cat, immediately after a Meal (11.0), and at various intervals afterwards (12.0, 2.0, 3.30, 4.30). (W. B. CANNON.)
c. Situation of œsophageal opening.
yz. 'Transverse band.'
wx. Junction of body and pyloric portions.

Movements may be observed even in a stomach which has been excised and placed in warm salt solution. They must therefore have their origin in the walls of the stomach itself. Although the co-ordination between the tonic contractions of the fundus and the rhythmic contractions of the pyloric part, may be carried out by the local nervous system—Auerbach's plexus—situated between the layers of the muscular coat, it is probable that the advancing waves of contraction observed in the antrum are myogenic, *i.e.*

* WILSON. *Can. Med. Assoc. Journ.*, 1937, **36**, 15.
† MESCHAN and QUIGLEY. *Amer. J. Physiol.*, 1938, **121**, 350.
‡ M'CREA, MCSWINEY *et al.* *Q. J. Exp. Phys.*, 1924, **14**, 379.

directly originated in and determined by the muscle fibres themselves. Cannon has shown that these movements persist after complete division of

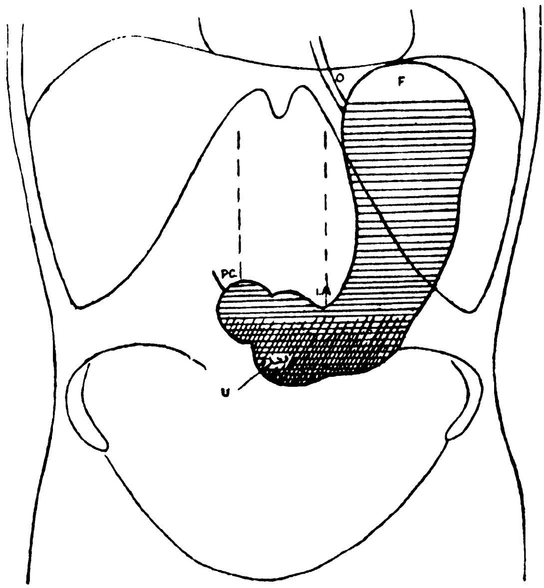

FIG. 548. Sketch of Human Stomach, in Erect Position, shortly after a Bismuth Meal. (HURST. *Quart. J. Med.*, 1910, **3**, 374.)

F. Fundus. U. Umbilicus. IA. Incisura angularis.
PC. Pyloric canal. O. Œsophagus.

Auerbach's plexus by from two to six circular incisions carried through the muscular coat of the stomach.

The influence of the nervous system on stomach movements is undoubted, since emotional states or unpalatable food lead to suspension or reduction in the tone and movements of the stomach, a fact which should be borne in mind in interpreting X-ray findings after barium meals. Stimulation of the peripheral end of the vagus nerves may exercise varying effects on the gastric wall as well as on its sphincters. In the normal animal, stimulation of the peripheral end of the vagus as a rule causes strong contractions of the œsophagus as well as of the cardiac sphincter. After the administration of atropine, stimulation of the same nerve will occasion dilatation of the cardiac sphincter. On both cardiac and pyloric portions of the stomach the vagus exercises inhibitory as well as augmentor effects. So far as concerns the musculature of the fundus or body of the stomach, the most usual result is an inhibition during stimulation of the vagus, succeeded by an augmented tonus immediately the stimulus is removed. If the vagus be excited a number of times, the tonus of the muscular wall augments with each stimulus. On the pyloric portion, stimulation of the vagus also causes inhibition, followed by contraction and augmented peristaltic waves. McSwiney and Wadge * found that the effect of vagus stimulation on the stomach as a whole depends on its state of tonus. When the tonus is low, stimulation of the vagus causes contraction; when it is high, relaxation results. In the pylorus itself we may obtain from vagal stimulation either increased or diminished contraction.

Whether the splanchnic nerve has a direct influence on the movements of the stomach has been disputed. Most observers regard the splanchnic as having an influence on the stomach similar to its action on the intestine, and regard it as the chief inhibitory nerve to this organ.

* McSWINEY and WADGE. *J. Physiol.*, 1928, **65**, 350.

Emptying of the Stomach. The pyloric sphincter is normally relaxed, and the pressures in the stomach and duodenal bulb are equal in the fasting stomach. If fluid alone be swallowed, as when water is taken to quench thirst, it may arrive in the duodenum within a minute or two after being swallowed. When food is in the stomach, the pressure within it is generally slightly above that in the duodenal bulb.* As peristaltic waves pass along, the intragastric pressure first rises and then falls.

When a meal is undergoing digestion, there is a distinct, though possibly fortuitous, relation between the amount of acid present in the gastric contents and the opening of the pylorus, but proteose and peptone are more important than acidity.† It has been shown by Campbell that in certain cases, otherwise normal, there may be complete achlorhydria, *i.e.* absence of free HCl from the gastric juice; and yet the movements and emptying of the stomach may present nothing abnormal. It appears more likely that the onward passage of gastric contents depends on two factors, first, the relaxation of the pyloric sphincter, and second, the relative pressures in the pyloric antrum and the first part of the duodenum (duodenal cap). When a wave in an intense phase reaches the pyloric antrum, the sphincter being relaxed, and the pressure in the antrum high, its contents escape into the duodenum. The contraction wave then involves the sphincter and closes it.‡ The normal emptying of the stomach is also largely dependent on the integrity of the connection of this viscus with the central nervous system. Hence if in a dog both vagus nerves be divided below the point at which they give off their branches to the lungs and heart, a large amount of food may remain in the stomach in an undigested condition. The secretion of gastric juice is deficient, and the opening of the pylorus is not easily carried out.

Liquids leave the stomach earlier than solid material; an increase of duodenal pressure causes increased tone in the pyloric sphincter and so retards emptying of the stomach, while a lowering of duodenal pressure has the reverse effect.§ After larger meals the emptying time is greater in most subjects than after small meals, *e.g.* doubling a small test meal may increase the time by 17 per cent., and trebling it may increase it by 38 per cent.‖

Meals of different foodstuffs remain in the stomach for different periods. Carbohydrates leave early, whereas in presence of fats there is longer retention, the delay being proportional to the quantity of fat,¶ and dependent also on its nature.** The inhibitory effect of fat on the stomach movements is produced when fat enters the upper intestine, and not while it is confined to the stomach; it appears to be partly reflex and partly humoral in origin.†† Towards the end of digestion a regurgitation of duodenal contents into the stomach will occur if the pressure is higher in the duodenum than in the antrum. The normal pressure in the duodenum is about 15 cm. water, and the pyloric intragastric pressure only 10 cm. during relaxation, but 20 to 30 cm. during an intense wave.

* THOMAS. *Amer. J. Physiol.*, 1938, **123**, 201.

† VAN LIERE, SLEETH and NORTHUP. *Amer. J. Physiol.*, 1937, **119**, 480.

‡ WHEELON and THOMAS, *Amer. J. Physiol.*, 1922, **59**, 72; MCSWINEY and PYRAH, *loc. cit.*

§ SPURRELL. *J. Physiol.*, 1935, **84**, 4.P.

‖ CRIDER and THOMAS. *Amer. J. Physiol.*, 1938, **123**, 44.

¶ MCSWINEY and SPURRELL. *J. Physiol.*, 1935, **84**, 41.

** ROBERTS. *Q. J. Med.*, 1930, **14**, 133.

†† QUIGLEY ZETTELMAN and IVY. *Amer. J. Physiol.* **1934**, **108**, **643**.

The contractions of the duodenum itself play an important part in regulating the emptying of, and regurgitation into, the stomach. Forward contractions empty the duodenal cap and facilitate exit from the stomach. Anti-peristalsis also occurs and, by filling the duodenal cap, gives an opportunity for regurgitation (Bolton).

The exact parts played in this mechanism by the local system and by the central nervous system respectively have not yet been thoroughly made out, though there is no doubt that some movements may proceed independently of any connection with the central nervous system.

When the stomach is empty the pylorus is kept relaxed, admitting of the free entry of duodenal contents (pancreatic juice and bile) into the stomach. But this regurgitation, especially of pancreatic juice, seems to be a frequent phenomenon even during digestion, and particularly when the meal contains fat, and to be a means by which the acidity of the gastric contents is prevented from rising above an optimum of 0·15 to 0·2 per cent. HCl.

Fractional examination of the gastric contents shows that, normally, the acidity reaches a maximum and then declines; after the decline has begun, bile is present in the fractions, showing that regurgitation has occurred (Fig. 549). If regurgitation is prevented in any way, the contents do not show the declining acidity, and no bile is found (Bolton).

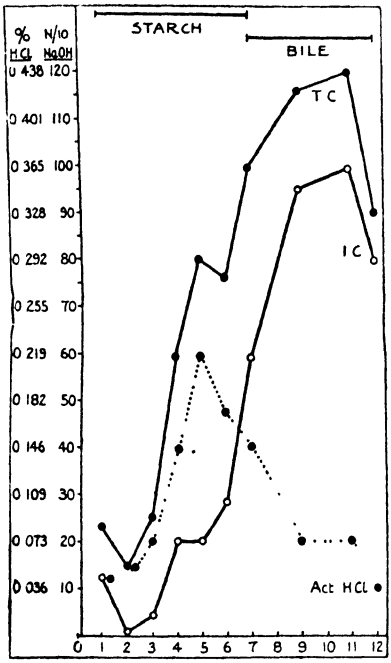

FIG. 549. Analysis of Gastric Contents at 15 min. intervals after a Test Meal of Gruel TC, total chlorides; IC, inorganic chlorides. Act.HCl, 'active' hydrochloric acid. (BOLTON.)

The work of Apperly suggests that the chief factor in emptying is the osmotic pressure of the gastric contents; the nearer this approaches to that of the saline constituents of the plasma, the more readily does the pyloric sphincter open; the duodenum only accepts chyme possessing approximately this osmotic pressure, otherwise regurgitating it back into the stomach.

The '*pangs of hunger*' are associated with, and are probably due to, prolonged rhythmic contractions of the stomach wall, which come on as soon as the stomach is empty, and increase in force and frequency; each contraction lasts about 20 seconds, and is accompanied by salivation and augmentation of the knee-jerks.* The hunger contractions are inhibited by the introduction of food into the stomach, by the act of swallowing and also by intravenous injection of glucose. Administration of insulin, which lowers the blood sugar, augments the contractions and causes hunger pangs.†

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* CANNON and WASHBURN, *Amer. J. Physiol.*, 1912, **29**, 441; CARLSON, "The Control of Hunger in Health and Disease," 1916. (Chicago Univ. Press.)

† CARLSON and QUIGLEY. *Amer. J. Physiol.*, 1931, **97**, 107.

VOMITING

Rejection of the stomach contents may occur as a result of over-distension of this organ, especially of the pylorus, of the presence of irritating material in its contents, or reflexly from various organs. It is generally preceded by a feeling of nausea, associated with excessive salivation. The large quantities of saliva swallowed still further distend the stomach and assist the opening of the cardiac orifice. In the act of vomiting itself, the first event is a deep inspiration. The glottis is then closed and the nasal chamber often shut off by raising of the soft palate, and this is followed by a strong contraction of the diaphragm and of the abdominal muscles. At the same time the œsophagus, the body of the stomach and the cardiac orifice are relaxed. By means of X-rays it is seen that at this time a strong contraction occurs at the incisura angularis, dividing the stomach into two separate portions. The dilated body of the stomach is pressed between the abdominal muscles and the diaphragm, so that its contents are expelled first into and then through the relaxed œsophagus and out through the mouth. Contraction of the body of the stomach may be slight or absent and the main factor in the expulsion is the contraction of the abdominal muscles and diaphragm. In fact, vomiting may be excited in an animal in which the stomach has been replaced by a bladder. At its close the œsophagus is emptied, partly by the positive pressure in the chest as a result of the action of the abdominal muscles, partly by contraction.

Nervous Mechanism of Vomiting. Normally the action of vomiting is reflex. It can be excited by tickling the back of the throat, when the afferent nerves are the trigeminal and the glossopharyngeal, or by irritation of the stomach, through the afferent fibres of the vagus. But it may be excited reflexly from almost any of the abdominal viscera, *e.g.* uterus, kidney, intestines, &c., or through the labyrinth or eyes, as is supposed to be the case in the vomiting of sea-sickness, and it is a marked symptom in many cases of disease of the cerebrum and cerebellum. The efferent impulses are carried by the vagi to the stomach, by the phrenics to the diaphragm, and by the various spinal nerves to the abdominal muscles. There are also inhibitory impulses descending the vagi to the œsophagus and cardiac sphincter. The reflex act depends on the integrity of the medulla, so that a ‘ vomiting centre ’ is sometimes said to be situated in the medulla.

Drugs may produce vomiting either by irritating the stomach, *e.g.* mustard and water, zinc sulphate, ipecacuanha, or by direct action on the medullary centres, *e.g.* apomorphine, &c.

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5. INTESTINAL DIGESTION

The contents of the stomach are passed at intervals into the first part of the duodenum. Here they meet the secretions of the pancreas, the liver, the tubular glands of the intestine, and the secretion of Brunner's glands. The acidity is partly neutralised in the duodenum, the contents of which have a pH of 4·5 to 5·0 ; further reduction of acidity occurs lower down in the intestine, the pH in the ileum being about 6·0 to 6·5, but actual alkalinity occurs rarely if at all.

The secretion of these various juices is simultaneously aroused by the entry of the acid chyme into the duodenum. Although they co-operate in

their action on the foodstuffs, it will be convenient to deal separately with each.

Duodenal Contents. Samples of the duodenal contents may be obtained in man by passing an Einhorn's duodenal tube, a 30 in. long narrow rubber tube with a small lead plummet embedded at the end. This is swallowed, and when the plummet reaches the stomach the patient lies on the left side until it reaches the duodenum, where its position can be verified by X-ray examination. By suction with a syringe the contents, frothy, and yellow, are withdrawn. If 20 c.c. of 50 per cent. $MgSO_4$ is injected down the tube a copious flow of bile follows.

THE PANCREATIC JUICE

THE SECRETION OF PANCREATIC JUICE. The normal secretion of pancreatic juice can be studied on an animal with a pancreatic fistula.

In order to establish a pancreatic fistula in a dog, a small piece of the duodenal wall is exsected, having the papilla of the lower (and larger) duct opening in the middle of its mucous surface. The integrity of the gut is restored, and the exsected piece is brought to the surface and stitched in the middle of the abdominal wound. The greater part of the pancreatic secretion will escape by the fistula, and can be collected. The continual loss of pancreatic juice may seriously affect the animal's health. This may be obviated to a certain extent by keeping the animal on a milk diet with the addition of sodium bicarbonate to replace the loss of this salt by the juice.

Clinical observations of human pancreatic fistulæ have confirmed the experiments made on dogs.

In the fasting condition there is for long periods (one to two hours) no expulsion of juice from the duct. Probably, however, there is occasional slow secretion of juice, coincident with a phase of spontaneous periodic activity of the alimentary canal (Babkin), so that the escape of a few drops may be observed at long intervals. If a meal be administered, a flow of juice begins in one to two minutes. This first secretion is reflex. From this time there is a steady, slow rise in the rate of secretion, which lasts for two to three hours, and then gradually diminishes. The greatest increase in flow is observed at the time when the first portions of digested food escape from the stomach into the duodenum. The secretion must therefore be determined in some way by this entry into the duodenum. By experiments on dogs possessing a gastric as well as a pancreatic fistula, it has been shown that the introduction of acid, *e.g.* 0·4 per cent. HCl, into the stomach evokes, as soon as it passes into the duodenum, a rapid flow of pancreatic juice. A smaller effect is produced by the passage of oil from the stomach into the duodenum. The introduction of alkalies is without influence. Dilute acids are also effective exciters of secretion when introduced directly into the duodenum or small intestine. The secretion produced diminishes as the loop employed is nearer to the cæcum, and as a rule the injection of dilute acid into the lower foot or eighteen inches of ileum is without effect. This response to acid is not due to a reflex, but to a humoral (chemical) mechanism.

Nervous Secretion. The pancreas receives fibres from the vagi and from the splanchnic nerves. Pavlov noticed that if the vagus on one side were cut in the neck and left for four days in order to allow the cardio-inhibitory fibres to degenerate, prolonged stimulation of the peripheral end of the nerve evoked a slow flow of pancreatic juice. He obtained the same results by stimulating this nerve below the heart, in animals with the cervical spinal cord divided. Pancreatic secretion resulted from vagus stimulation when every care was taken to prevent acid stomach contents being expelled into the duodenum by contraction of the stomach wall; *e.g.* when the pylorus

was ligatured and the stomach filled with an alkaline solution. There can be no doubt that the vagus is a secretory nerve to the pancreas, and that the secretion of pancreatic juice which occurs within a minute or two of the taking of food is a reflex. The juice obtained by vagus stimulation is glairy, very concentrated in solids, and rich in enzymes. When secretion has been established by prolonged and repeated stimulation of one vagus, a stimulation of the other vagus at first checks it. This is due to constriction of the ducts. (ANREP).* A slight secretion, inhibited by atropine and potentiated by eserine, also occurs on stimulation of the splanchnic nerves, but not on injection of adrenaline. The splanchnic therefore contains some cholinergic fibres.†

Humoral Secretion. The injection of acid into a loop of small intestine is followed by secretion of pancreatic juice. Bayliss and Starling ‡ found that a secretion of juice was produced even when the acid was introduced into a loop of the small intestine entirely freed from any possible nervous connections with the rest of the body. It was evident, therefore, that the stimulus from the intestine to the pancreas, which causes the secretion of the latter, must be carried, not by the nervous system, but by the blood stream. The injection of acid into the portal vein was without effect, but on boiling some scrapings of the intestinal mucous membrane with dilute hydrochloric acid, neutralising and filtering, and injecting the filtrate a copious flow of pancreatic juice was produced. This classical experiment provided the first known instance of a chemical excitant, and the name ‘ hormone ’ was given to this class of substances.

Secretin. This chemical messenger, or hormone, is called ‘secretin.’ That it is present in the mucous membrane is shown by the fact that secretin can be extracted also by water, solutions of soap, or dilute alkali, or by the prolonged action of alcohol upon mucous membrane which has been killed by heat. Neutral solutions, however, extract less secretin than do acid solutions. This may be because some of the secretin is held in an adsorbed or otherwise ‘ bound ’ form, from which it is freed by acids.§

Highly active preparations of secretin may be prepared in various ways. One || is by extraction of the duodenal mucosa with absolute alcohol, precipitation by dilute acid, and resolution of the precipitate in acid alcohol and precipitation by acetone. Various other methods have been used and crystalline preparations ¶ ** may be obtained. Greengard and Ivy's crystalline secretin is obtained as a picrolonate of empirical composition C_3H_3ON ; 0·014 mg. of the free secretin from this = 1 Ivy-dog-unit, *i.e.* it gives 1 c.c. of juice. It appears to be a compound of low molecular weight, and is a readily soluble substance resembling a polypeptide, is rapidly destroyed by pepsin and trypsin, and diffuses slowly through animal membranes. Though stable in acid solutions, it is very rapidly destroyed in alkaline or neutral solutions, especially under the influence of bacteria.

In this secreting mechanism we have an example of a correlation effected by chemical means, between the activities of two different portions of the body. The acid chyme enters the first part of the duodenum. Immediately a certain amount of secretin is extracted by the acid, is carried by the blood stream to the cells of the pancreas and excites there the secretion of strongly alkaline pancreatic juice. As soon as sufficient juice has been secreted

* ANREP. *J. Physiol.*, 1916, **50**, 421.
† BABKIN *et al.* *Q. J. Exp. Physiol.*, 1939, **29**, 217.
‡ BAYLISS and STARLING. *J. Physiol.*, 1902, **28**, 325.
§ SCOTT and STILL. *Amer. J. Physiol.*, 1935, **112**, 511.
|| J. MELLANBY. *Proc. R. S.*, 1932, **111B**, 429.
¶ HAMMARSTEN *et al.* *Acta. Med. Skand.*, 1928, **68**, 1.
** GREENGARD and IVY. *Amer. J. Physiol.*, 1938, **124**, 427.

to nearly neutralise the acid chyme, the formation of secretin, and therefore the further secretion of alkali, comes to an end. If the stomach still contains food, however, the process is renewed, the pylorus relaxes and allows the entrance of a further portion of acid chyme. Thus the whole chain of processes goes on until the stomach is empty and all its contents have passed into the intestine. One of the actions of the powerful secretin preparations made by Mellanby is that it causes contractions of the intestinal muscle; it also causes some bile secretion.

An important part appears to be played by the presence of the bile salts in the intestine, since Mellanby found that the introduction of bile, or bile salts, together with acid, gives rise to a particularly profuse secretion; the secretin and bile salts are possibly absorbed together.*

The secretin mechanism provides alkali for the neutralisation of the acid stomach contents. But the nervous mechanism provides the enzyme content of the juice, so that, even when for any reason the gastric contents are not acid, pancreatic digestion is still possible.†

In the absence of HCl from the gastric contents, it is probable that the bile is the responsible agent in starting the secretin mechanism.

The amount and activity of pancreatic juice obtained after a meal varies with the nature of the latter. The differences seem largely determined by the amount of acid secreted in the stomach and passed on to the duodenum, and by the presence of specific excitants, such as fat and soap, which raise the enzyme content of the juice. There does not seem to be any qualitative alteration in the constitution of the juice such as to adapt the secretion to the food. Apparently when the pancreas is excited to secrete, it turns out its various enzymes in constant proportion, depending on the amounts of these already present and stored up in the gland.

Electrical Changes. Stimulation of the pancreas by secretin injection causes electrical changes in which the duct of the pancreas becomes positive to the surface of the gland ‡

For Reference

STILL (1931). Secretin. *Physiol. Rev.*, **11**, 328.

	A	B (a)	B (b)	C
Alkalinity:				
Number of c.c. $\frac{N}{10}$ NaOH equal to 10 c.c. juice	12·7	12·4	9·0	5·5
i.e. in terms of Na in 100 c.c.	0·29	0·28	0·20	0·12
Total solids in 100 c.c.	1·6 1·56	2·25	1·5	6·38 6·40
Total proteins in 100 c.c.	0·5	—	—	4·8
Ash in 100 c.c.	1·00 0·92	1 00	1 00	1·3
Chlorides in 100 c.c.	0·28 0·30	—	—	0·27
Total nitrogen	—	—	—	0·74

A. Secretin juice from three dogs. Sp. gr. 1014.
B. Secretin juice, specimen collected at beginning (*a*), and at end (*b*).
C. Pilocarpine juice.

* J. MELLANBY. *J. Physiol.*, 1926, **61**, 419.
† J. MELLANBY. *J. Physiol.*, 1925, **60**, 85; *Proc. Roy. Soc.*, 1932, **111B**, 429.
‡ ANREP and DALY. *J. Physiol.*, 1921, **55**, ii, Proc.

The Structural Changes in the Pancreas accompanying Secretion

A normal resting gland on section is seen to consist of numerous secreting alveoli, he cells of which present two well-marked zones: a narrow peripheral zone in which he nucleus is embedded, and which is strongly basophile; and a central zone which s turned towards the lumen, occupying two-thirds or three-quarters of the cell, and is losely packed with highly refractive granules, strongly oxyphile, and containing the ymogens of the pancreatic juice (Fig. 550 A). After the induction of secretion by timulation of the vagus, the lumen of each alveolus is enlarged, the cells are shrunken, nd the granules are found to lie only along the border of the cell turned towards the imen, the rest of the cell, which is much reduced in size, being made up of the basophile ytoplasm (Fig. 550 B). After secretion has been caused by secretin injection, the hanges are only slight.

PROPERTIES OF PANCREATIC JUICE. Pure pancreatic juice an be obtained, from an animal with either a permanent or temporary fistula,

A B

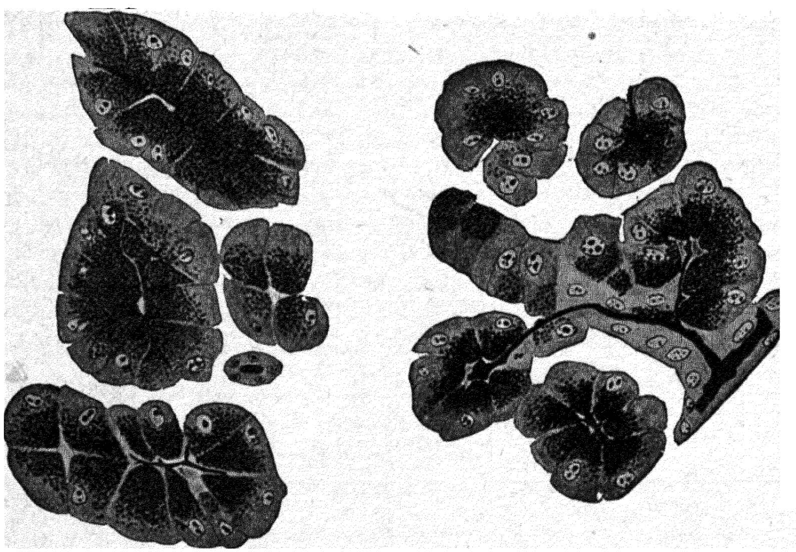


FIG. 550. Alveoli of Dog's Pancreas. (BABKIN, RUBASCHKIN and SAVITSCH, *Arch. Mikr. Anat.* 1909, **74**, 68.)
A. Resting. B. After moderate secretion with discharge of granules.

y the injection of secretin or pilocarpine intravenously, or by stimulation f the vagus. The average composition of pancreatic juice is shown in the Table on p. 913.

'Secretin' juice is a clear or slightly opalescent fluid, alkaline (pH = 8·7—·9) from the presence of sodium bicarbonate, its titratable alkalinity varying etween $\frac{N}{10}$ and $\frac{N}{7}$ alkali. It is therefore about as alkaline as gastric juice is cid. The proteins of the juice may be roughly divided into three groups, a mall amount of nucleo-protein precipitated on acidification, a protein oagulating at 55° C., and another at about 75° C. The juice tends to become oor in proteins and enzymes, but not in alkali, as secretion proceeds. The oncentrated juice obtained by injection of pilocarpine, or by vagus timulation, which may contain as much as 6 per cent. total solids, is lways considerably less alkaline and richer in enzymes than the more dilute uice got by injection of secretin.

The most interesting and important constituents of the juice are its enzymes. The juice on arrival in the intestine has, or develops, an effect on all three classes of foodstuffs: proteins, fats and carbohydrates, due to the presence of distinct groups of enzymes, viz. trypsin, lipase and amylase.

The Action of Pancreatic Juice on Proteins. The digestive action of pancreatic juice on proteins was discovered by Kühne, who in 1876 investigated the action of extracts of the gland. The failure of Claude Bernard to observe this action must be ascribed to the fact that he worked with pure pancreatic juice, since for the development of this power it is necessary that the trypsin be activated. Activation under normal circumstances is effected directly the juice enters the gut, by the action of *enterokinase* contained in the succus entericus.* The pancreatic juice in the body thus acquires a proteolytic activity superior to that of any other digestive juice.

These enzymes may be separated from one another, and so studied separately, by appropriate adsorption treatment as explained in Chapter IX.

Inactive pancreatic juice is now known to contain two zymogens, viz. chymotrypsinogen and trypsinogen.† Enterokinase converts trypsinogen to trypsin, but does not act on pure chymotrypsinogen. This is, however, acted upon by trypsin and converted into chymotrypsin. Hence, enterokinase added to a mixture of the two zymogens results in activation of both. Chymotrypsin clots milk, and hydrolyses casein and gelatine, but differently from trypsin; it does not hydrolyse polypeptides in general.

Trypsin acts on proteins generally, converting them to polypeptides.

It is now known that 'trypsin' is a mixture of several proteolytic enzymes, *e.g.* trypsin, aminopolypeptidase, carboxypolypeptidase, protaminease, and no doubt others. It also contains erepsin and a substance which inhibits trypsin. These enzymes act in different ways, or on different substrates. Thus carboxypolypeptidase does not act on proteins, but only on some of their hydrolysis products, from which it splits off a terminal amino acid which in the intact substrate had a free carboxyl group. The free amino group is not necessary and hence carboxypolypeptidase will act on peptic digests which have been treated with formaldehyde to remove free NH_2 groups.‡ Aminopolypeptidase only acts on polypeptides with free α-amino groups, splitting off the amino-acids.

The different constituents of the protein molecule show a varying resistance to the action of trypsin. The greater part of the molecule is rapidly broken down and yields proteoses, peptones, polypeptides, and amino-acids; and the same change is undergone by the proteoses and peptones resulting from the gastric digestion of proteins. Within a few minutes, therefore, after the chyme has reached the small intestine, a certain amount of amino-acids will have been formed. Trypsin thus differs fundamentally in its action from pepsin, which never yields amino-acids. Some of the products present a resistance to disintegration. This part gives the biuret reaction. Even when the trypsin has acted *in vitro* for weeks, and the biuret reaction has entirely disappeared, the mixture will be found to contain, in addition to the separate amino-acids, some lower polypeptides not attacked by pure trypsin.

The action of trypsin is thus seen to resemble very closely the early action of boiling concentrated hydrochloric acid. Like the latter it attacks the protein molecule at the—CO—NH—coupling, introducing water at this point and therefore breaking off simple amino-acids. In all, activated trypsin alone can split about half the peptide linkages in native protein.

* SCHEPOWALNIKOV. *Jahresb Fortschr. Tierchem*, 1900, **29**, 378.
† KUNITZ and NORTHROP. *J. Gen. Physiol*, 1935, **18**, 433.
‡ ANSON. *Ergebn. d. Enzymforschung*, 1938, **7**, 118.

Why it always leaves the simple polypeptides unattacked is not at present explained. The investigation of its action on the higher polypeptides has shown that very minute differences in the grouping of the molecule may determine whether or not the molecule is attacked by trypsin.

CONDITIONS OF TRYPTIC ACTIVITY. Since the alkaline pancreatic juice when secreted meets the correspondingly acid contents discharged from the stomach, the resulting mixture in the duodenum is faintly acid (pH 4·5—5·1). *In vitro*, trypsin is more active in alkaline solutions. The optimum pH varies with the substrate, *e.g.* 9·7 for gelatin and 8·0 for caseinogen and peptone. However, trypsin in alkaline medium is extremely unstable, so that, when prolonged digestions are carried out, the trypsin would be rapidly destroyed if the medium were strongly alkaline. The destructibility of trypsin, as well as its action, is largely affected by the presence of proteins or their digestion products in solution. Bayliss adduced evidence that trypsin enters into some form of combination with the protein molecule. This combination protects the trypsin from the destructive action of alkali. Destruction of the enzyme nevertheless occurs in the intestine, and intestinal contents collected at a fistula at the lower end of the ileum show little or no proteolytic activity.

THE ACTIVATION OF PANCREATIC JUICE. Freshly secreted pancreatic juice, collected without contact with the intestinal mucosa, has no digestive action on egg-white, though able to digest peptone, histone or protamines. Succus entericus very rapidly activates it, owing to the presence in it of *enterokinase*. Enterokinase acts like an enzyme * on the inactive enzyme (trypsinogen) to form the activated enzyme, trypsin. Trypsinogen has been prepared in pure crystalline form,† and is rapidly activated by enterokinase at about pH 5·5.

Most of the controversy as to whether enterokinase is an enzyme appears to be due to the use of crude preparations.‡ Pure crystalline trypsinogen is autocatalytically activated by the minutest trace of trypsin, the change being due to some internal molecular rearrangement. Crude preparations, however, contain an inhibitor of trypsin which checks this reaction, so that normally only the kinase can start it, and this is the state of affairs in the alimentary tract, where activation occurs in a few seconds. Activation can also be brought about by an enzyme formed by the mould *penicillium*, at pH 2·5-4·0.§

If pancreatic juice be allowed to stand, it gradually acquires activity. If, however, sodium fluoride be added, the juice remains permanently inactive. The spontaneous activation of the juice may be hastened by neutralisation. The most potent means next to enterokinase is the addition of calcium salts. According to J. Mellanby and Woolley ‖ the calcium acts simply by neutralising the juice and thus allowing minute traces of kinase already present in the juice to exert their effect. It might thus act by diverting the trypsin inhibitor.

THE ACTION OF PANCREATIC JUICE OF MILK. On the addition of activated pancreatic juice to milk, a clot is produced which speedily redissolves. The inactive juice has no milk-clotting action. The enzyme to which the effect is due is called *chymotrypsin*, and it closely resembles trypsin, with which it is associated. In fact, some authorities consider the milk-clotting action to be one of the properties of trypsin. The two enzymes have, however, been separated in crystalline form.

* VERNON, *J. Physiol.*, 1901, **27**, 269; 1913, **47**, 325. KUNITZ, *J. Gen. Physiol.*, 1939, **22**, 429.

† KUNITZ and NORTHROP. *J. Gen. Physiol*, 1934, **17**, 591.

‡ NORTHROP. *Science*, 1937, **86**, 479.

§ KUNITZ. *J. Gen. Physiol.*, 1938, **21**, 601.

‖ MELLANBY and WOOLLEY. *J. Physiol.*, 1913, **46** 159.

The Action of Pancreatic Juice on Carbohydrate. The pancreatic juice, as well as a fresh extract of the pancreas itself, contains a strong amylolytic enzyme, amylase or amylopsin (optimum pH = 7·0). The stages in the hydrolysis of starch brought about with pancreatic juice are exactly similar to those effected by ptyalin.

If the juice be neutralised, or faintly acidified, the process of hydrolysis goes on to the formation of glucose.* This is due to the presence in the juice of a second enzyme—maltase—which converts maltose into glucose. The juice in the gut is therefore able to effect the further digestion of the products of salivary digestion. On the other disaccharides pancreatic juice is without effect.

The Action of Pancreatic Juice on Fats. Fresh pancreatic juice contains a powerful esterase, called *lipase*, by means of which, in the presence of water, neutral fats are broken up into glycerol and the corresponding fatty acids. This enzyme is active either in an alkaline, neutral or very slightly acid medium (optimum at pH = 8·0). It also hydrolyses various other esters besides fats. If the reaction be alkaline, the fatty acids produced by the lipolysis combine with the alkali present, with the formation of soaps. The enzyme may be obtained from extracts of the fresh gland, but is rapidly destroyed if active trypsin be present. It is also contained in some of the dried commercial preparations of trypsin. It is insoluble in distilled water, but easily soluble in glycerol. The velocity with which lipolysis occurs is much increased (four to five times) by the addition of bile. This adjuvant action of bile is due entirely to the bile salts. These act in two ways: In the first place, by their physical qualities they diminish the surface tension between water and oil, so causing the fat to break up into fine droplets which expose a large surface to the action of the lipase; emulsification is also aided by the presence of soaps; it is important because fat is insoluble and only attacked at the surface. In the second place, bile salts have a solvent action on calcium soaps and on fatty acids in slightly acid medium. Bile may be regarded, therefore, as a favourable medium for the interaction of the lipase and the neutral fats.

6. THE LIVER AND BILE

In the adult, the relation of the liver cells to the ducts is entirely subordinated to their relation to the blood vessels of the organ, and it requires special histological methods to make out the relations between the liver cells and the bile ducts. The circulation through the liver is described in Chapter XXXV.

Liver tissue is very rapidly removed if it becomes necrotic as, *e.g.*, after occlusion of a branch of the hepatic artery or after chloroform poisoning; it rapidly grows again after destruction or removal and regains its normal size. Even after extirpation of three-fourths of the liver the remainder soon recovers its normal size, though it is of softer texture. The liver varies greatly in size according to the state of digestion, and in fasting may be only half the normal size. The size of the liver is very delicately adjusted, and if after removal of part of the liver the animal be fasted, recovery growth ceases when the size of the fasting liver has been reached. (Rous.)

As might be expected from its structure, the secretory functions of the liver represent but a small proportion of its activities in the body. The liver is in fact the greatest chemical factory of the body, receiving by

* Mellanby and Woolley. *J. Physiol*, 1914, **49**, 246.

the portal vein the products of digestion as they are absorbed from the alimentary canal. It converts these into other substances, breaking them down or building them up according to the needs of the body as a whole. Hence the liver plays an important part in the metabolism of proteins, carbohydrates and fats, so that its functions will have to be dealt with in the various chapters concerned with the fate of the different foodstuffs in the body. In this chapter we are merely concerned with its action as a secreting gland, in forming the bile.

THE BILE

In addition to its function in digestion, the bile must also be regarded as an excretion, representing, as it does, the channel by which the products of disintegration of hæmoglobin are got rid of from the organism. As an excretion, the production of bile must be continuous and not necessarily related to the processes of digestion. On the other hand, bile as a digestive fluid is needed in the gut only during the period that digestion is going on. The exigencies of the body therefore require a continuous excretion, but a discontinuous entry of this fluid into the small intestine. This is secured, in the majority of animals, by the existence of the gall bladder, in which all bile, secreted during the intervals between the periods of digestive activity, is stored up. In the horse and the rat, where the gall bladder is absent, its place is taken to some extent by the great size of the bile ducts. Since the bile accumulates in the gall bladder during the whole time that digestion is not going on, and is only poured into the gut during digestion, in a fasting animal the gall bladder is distended, whereas some hours after a meal it is less full.

The common bile duct and one of the pancreatic ducts open together into the duodenum in man, the ape, horse, cat and dog ; in other animals either the pancreatic duct opens into the bile duct (sheep, rat), or else the two open separately, the pancreatic duct being the further down the duodenum (ox, pig, rabbit, guinea-pig).

In structure the gall bladder and bile ducts have an incomplete layer of peritoneum on the outside ; the middle coat consists of plain muscle and fibrous tissue and the inner lining of tall clear columnar cells. The common bile duct runs obliquely, sometimes for a centimetre or two, through the duodenal wall before opening on its inner surface. This part forms an expanded portion, or ampulla, having a strong muscular wall, which is further reinforced near the opening by a circularly arranged layer of plain muscle fibres, the sphincter of Oddi.

The walls of the gall bladder and bile ducts secrete a fluid containing mucin, and also absorb water from the bile. When the cystic duct becomes obstructed the gall bladder ultimately becomes filled with a colourless mucilaginous secretion—the so-called "white-bile" of surgeons. During its stay in the bladder the bile is rapidly concentrated by the loss of water and by the addition to it of mucin or nucleo-protein, derived from the cells lining the bladder. This is shown by comparison of the composition of human bile obtained from the gall bladder with that obtained from a fistula of the bile duct. The speed with which the gall bladder concentrates the bile is remarkable, the total solids of bladder bile reaching as much as ten times that of fistula bile, and the volume showing a corresponding reduction.

The inorganic salts are mostly sodium chloride and bicarbonate, the fixed CO_2 of the bile varying from 10 to 60 volumes per cent. The reaction of liver bile in the hepatic ducts is usually slightly alkaline (*p*H 7·7), while in the gall bladder it is slightly acid (*p*H 6·8) as a rule.

ANALYSES OF BILE (HUMAN) [per cent.]

From a biliary fistula (Yeo and Herroun)		From the gall bladder (Czyhlarz *et al.*)	
Mucin and pigments	0·15	Mucin	1·64
Sodium taurocholate	0·06	Bile Pigments	0·16
Sodium glycocholate	0·16	Bile Salts	4·67
Cholesterol	0·04	Cholesterol	0·66
Lecithin		Lecithin	0·56
Inorganic salts	0·84	Inorganic Salts	0·85
Water	98·72	Water	90·57

From the point of view of digestion, the important constituents of bile are the '*bile salts*,' with the lecithin and cholesterol held in solution by these salts.

The Bile Salts. Characteristic of bile are the so-called "bile salts," which give the well-known Pettenkofer reaction—a purple colour with cane sugar and strong sulphuric acid. The bile salts are sodium salts of mixtures of complex acids, called *glycocholic and taurocholic acids*. Glycocholic and taurocholic acid are both present in human bile, but in the bile of the dog and the sheep there is no glycocholic acid. These acids themselves are compounds of one or more of a group of acids called cholic acids and can readily be resolved by hydrolysis with acids into their proximate constituents thus:—

Glycocholic acid = glycine + cholic acid
Taurocholic acid = taurine + cholic acid.

Taurine is amino ethyl sulphonic acid, $NH_2.CH_2.CH_2.SO_2.OH$, and is probably derived in the body from the metabolism of cystine.

The Cholic Acids (cholic acid, desoxycholic acid, chenodesoxycholic acid and lithocholic acid) on dehydration and subsequent reduction all yield *cholanic acid*, $C_{24}H_{40}O_2$. Cholanic acid or its isomer, allo-cholanic acid, can be obtained from cholesterol by reduction, and subsequent oxidation with chromic acid. It is thus clear that the bile acids are chemically closely related to cholesterol (see formula, p. 33).

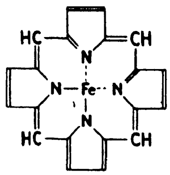
Though this is so, and although it is probable that the bile salts are produced in the liver, there is no conclusive evidence for this, and very little for the supposition that, in the body itself, cholic acid is actually derived from cholesterol.

Many substances, *e.g.* fatty acids, higher alcohols, etc., form loose compounds, called *choleic acids*, with desoxycholic acid, and as these compounds are soluble in water, the bile salts are capable of bringing into aqueous solution many normally insoluble substances. This is called "hydrotropic action."

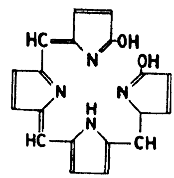
When bile salts are administered by the mouth they are excreted quantitatively in the bile. When cholic acid is administered, there is an increased excretion of bile salts, provided the necessary taurine is also given or made available, as is the case if a liberal protein diet is given at the same time. Even in fasting animals, however, there is a constant production of bile salts, though in smaller amount.

The Bile Pigments, *bilirubin* and *biliverdin*, are by-products, formed by the cells of the reticulo-endothelial system in various parts of the body from hæmoglobin of the blood (Ch. XXIX.). Injection of hæmoglobin into the blood stream, or extensive destruction of blood in the body, lead to an increased formation of bile pigment. The pigments thus formed circulate in small amount in the blood plasma from which it is excreted by the liver into the bile. They pass into the intestine, and are there converted by the processes

of bacterial reduction into stercobilin, which is excreted for the most part with the fæces, a small proportion being absorbed into the blood and turned



Hæm.

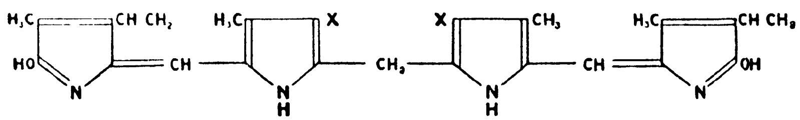


Bilirubin.

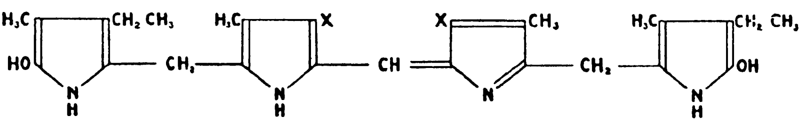
(Skeleton formulæ)

out as stercobilin in the bile or as urobilin in the urine. When the bile of a dog is all drained off by a biliary fistula, stercobilin disappears from the fæces and bile. If bile is given by mouth it reappears. When absorption of stercobilin is excessive, urobilin appears in the urine.

The Secretion of Bile. It is important to distinguish between the formation of the bile in the liver, which is a continuous process, and the expulsion of the bile, either direct or from the gall-bladder, into the intestine, which is discontinuous and principally dependent on the taking of food. The time-



Bilirubin



Urobilin ($X = -CH_2\,CH_2\,COOH$)

relations and other phenomena of the secretion of bile can be learnt from animals or men in which, by operation, the bile is conducted to the outside by means of a permanent fistula. Very little reliable information can, however, be obtained direct from men, and most of our information has been obtained from dogs. Of the various types of operation suited for the study of the bile flow, the best is probably one used by Rous and his colleagues, because it is carried out quite aseptically, and the animals can be kept in normal health for several months. The operation, called 'altercursive intubation,' used by McMaster and Elman, is of this type, and is illustrated in Fig. 551.

The secretion of the bile is independent of the action of the nervous system on the liver, since complete denervation of the liver leaves it unaltered and does not affect the influence of food on bile secretion.* Though the bile is always being secreted, the amount and concentration are both lowered during fasting. It is also reduced if the bile as it is formed is collected instead of being allowed to enter the duodenum. Bile formation also occurs independently of the action of the nervous system in an excised perfused liver.

If the hepatic duct be obstructed, as by attaching to it a manometer (Fig. 551) filled with bile, it is found that the flow continues at

* LUNDBERG, *Amer. J. Physiol.*, 1931, **98**, 602; HILLYARD, *ibid.*, 612.

a constant rate, so that the pressure rises uniformly, until a pressure of about 300 mm. of bile is reached, at which point it abruptly ceases. At this level re-absorption of the bile into the blood takes place.

The rate of bile secretion in man is believed to be about 15 c.c. per kg. body weight per day, or, say, about 800–1,000 c.c. for average adults; in dogs it varies in different individuals between about 5 c.c. and 20 c.c. per kg. per day. When food, especially meat or fat, is taken, the rate of secretion begins to increase in about an hour and reaches a maximum in two to five hours after the taking of food, then declining to the fasting rate. Carbohydrate food causes no increase. The secretion of the bile, after meat or fat, thus runs roughly parallel to that of the pancreatic juice.

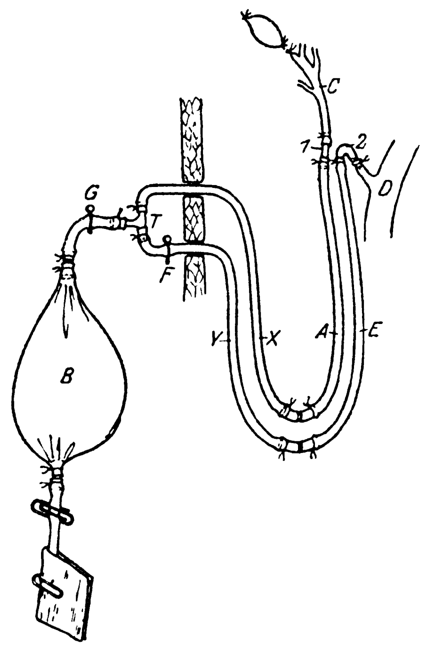


FIG. 551. Schema of the operation for "altercursive intubation." The bile can flow normally from *C* along the tubes *A*, *X*, *T*, *Y*, *E*, into the natural opening in the duodenum. When desired, *F* is closed and *G* opened, so that bile collects in the rubber bag *B*. A manometer can be connected to *X* to register secretion pressure, or a bile-filled manometer connected to *Y* to measure the resistance to flow of bile into the duodenum. The gall-bladder is excluded by ligatures. (MCMASTER and ELMAN, *J. Exp. Med.*, 1925, **41**, 513.)

Various substances other than foods cause an increased bile secretion when introduced into the stomach or duodenum, even after the division of all the nervous connections of the liver. The most potent of all these substances is bile itself, or the salts of the bile acids,* which powerfully excite bile formation and also increase the total solids of the bile formed. The active substance is cholic acid, which acts similarly when given by intravenous injection. The introduction of dilute acids into the duodenum, or the intravenous injection of ordinary secretin preparations also greatly augments the bile flow. It is uncertain by what means these various humoral agents act in promoting the formation of bile. The view of Bayliss and Starling that secretin stimulates the flow of bile as well as of pancreatic juice has been confirmed.† Probably an alteration in the blood flow to the liver is an important factor in modifying the rate of bile secretion.

When bile is introduced into the duodenum the bile salts are absorbed and re-excreted in the bile, as already explained, thus providing a so-called 'entero-hepatic circulation' of the bile salts. There is some loss of bile salts in the intestine, but this is made good by resynthesis if the animal is fed. The cat can re-form two-thirds of all the cholic acid in its body if this has been withdrawn by emptying the gall bladder during a fast.‡

There appears also to be a similar circulation of the bile pigments. Thus, if in a dog with altercursive intubation the bile is collected in the

* J. MELLANBY. *J. Physiol.*, 1928, **64**, 331.
† STILL, MCBEAN and RIES. *Amer. J. Phys.*, 1932, **99**, 94.
‡ MELLANBY and SUFFOLK. *Proy. R. S.*, 1938, **126**, 287.

bag for a time, the pigment content of the later samples secreted becomes reduced. If the bile be now returned to the duodenum, the pigment content of the subsequently secreted samples of bile is increased again. If the dog be fed with the bile of an herbivorous animal, the characteristic herbivorous pigment appears in the dog's bile. The presence of bile in the intestine seems to be essential to health. If the bile is all led away, bone defects are liable to occur, and a serious state of anæmia sets in ; the animal is restored to health again by being fed with bile, or with blood or blood pigment.

Bile Storage and Expulsion. The alterations occurring in the size of

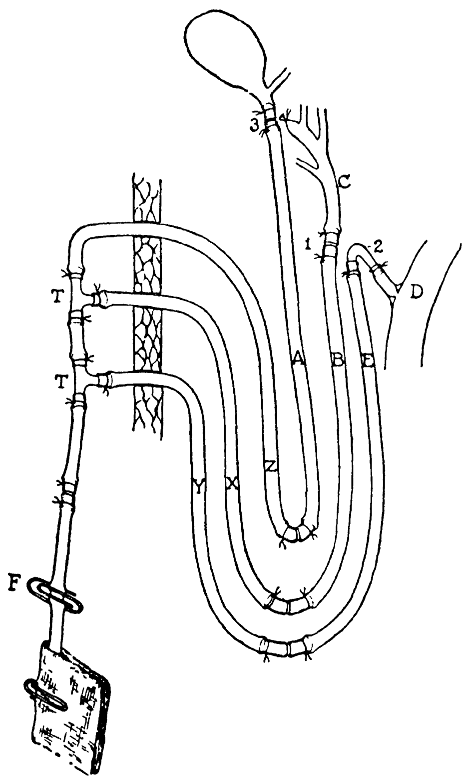


FIG. 552. Schema of triple intubation operation. In addition to the altercursive intubation there is a third tube, AZ, leading from the gall-bladder. When the system is clamped at F, bile flows normally. A bile-filled manometer connected to Y enables the resistance to bile entry into the duodenum to be measured ; a manometer connected to Z gives the pressure exerted by the gall-bladder, and one to X the secretion pressure of the bile. (McMaster and Elman, *J. Exp. Med.*, 1926, **44**, 184.)

the gall-bladder can be most readily observed clinically by the administration of tetra-iodo-phenolphthalein, which is excreted in the bile and is opaque to the X-rays, so that the outline of the gall-bladder can be seen on X-ray examination (Graham-Cole test).

The storage of bile in the gall-bladder and its expulsion therefrom are best investigated by simultaneously studying the pressure of bile in the common duct and the resistance to its entry into the duodenum. This may be done by the triple intubation operation of Elman and McMaster, shown in Fig. 552. The sphincter of Oddi is competent to close the entrance of the bile duct into the duodenum when the pressure of bile is low (below

150 mm. H_2O), and its action is further aided by contraction of the muscular wall of the duodenum itself.* After a fast of twenty-four hours, or on a carbohydrate diet, the resistance to the entry of bile into the duodenum is high, sometimes as great as 300 mm. H_2O. Hence the bile as it is secreted by the liver is diverted into the gall-bladder, and, since it is there very rapidly concentrated by loss of water, the storage of bile can continue for days without there being a great rise of pressure in the bile ducts, in which it is usually about 100 mm. H_2O during fasting. There is thus no entry of bile into the duodenum, since a pressure over 300 mm. would be necessary to force the sphincter.

When food is seen or eaten, there is at once a lowering of the sphincter resistance and at the same time a contraction of the gall-bladder great enough momentarily to raise the pressure in the duct system to about 200 mm. H_2O. This is probably assisted by a contraction of the ampullary part of the common duct. A gush of bile into the duodenum follows. Afterwards the resistance suddenly rises again, but in about half an hour another rise of pressure with relaxation of the sphincter occurs, and thereafter at fairly frequent intervals for the next few hours bile is expelled into the duodenum.

The control of the gall-bladder seems to be a twofold one—by a reflex and by a hormone. The muscular wall of the gall-bladder shows rhythmic contractions, and, as was shown by Bainbridge and Dale,† is under the control of nerves derived both from the vagus and from the sympathetic, the former conveying motor and the latter inhibitory impulses, while the sphincter of Oddi receives branches from the same sources, but having the opposite functions. It seems certain that the sight of food or the act of eating it, causes a reflex contraction of the gall-bladder, with reciprocal relaxation of the sphincter of Oddi. It has also been supposed that the entry of acid into the duodenum provokes a similar reflex,‡ and there is clear evidence that under these circumstances the resistance of the sphincter is reduced.

That much of the activity of the gall-bladder is due to the action of a hormone liberated into the blood by the presence of excitants in the duodenum seems equally certain. Fat and egg-yolk are the most active substances in this connection, and cause contraction even of the denervated gall-bladder. Ivy and Oldberg § have extracted from the duodenal mucosa a secretin-like substance, '*cholecystokinin*,' which stimulates contraction of the gall-bladder when injected.

Introduction of magnesium sulphate into the duodenum causes relaxation of the sphincter and expulsion of bile. Morphine causes strong spasm of the sphincter.

It is of interest to note that the expulsion of bile into the duodenum serves a twofold function. First, it promotes pancreatic secretion by aiding in the transference of secretin into the blood, and secondly, it augments the flow of the bile itself.

THE DIGESTIVE FUNCTIONS OF THE BILE. The digestive functions of bile are due, not to the presence of enzymes, but to the peculiar influence of the bile salts on the actions of the pancreatic juice. Most important is

* LUETH. *Amer. J. Physiol.*, 1931, **99**, 237.

† BAINBRIDGE and DALE, *J Phys.*, 1905, **33**, 138, WESTPHAL, *Z. f. klin. Med.* 1923, **96**, 52.

‡ OKADA. *J. Physiol.*, 1915, **50**, 42.

§ IVY and OLDBERG, *Amer. J. Physiol.*, 1928, **86**, 599; LUETH, IVY and KLOSTER, *ibid.*, 1929, **91**, 329.

the part played by the bile in the digestion and absorption of fats. The fat-splitting action of pancreatic juice is trebled by the addition of bile, whether boiled or unboiled. This quickening action of the bile probably depends on the hydrotropic action of the bile salts. Not only does this diminish the surface tension between watery and oily fluids, so promoting emulsification, and thus the closer approach of the lipase to the fats, but it involves dissolving fatty acids and soaps, including even the insoluble calcium and magnesium soaps. Once an emulsion of fat has been formed with the aid of the bile salts, this is stabilised by various accessory agents, mucin, soaps, proteoses, &c.

Further, the bile salts are activators of pancreatic lipase, even when this acts on substances in solution. It is possible that this activating effect is due to the bile salts in some way bringing the enzyme and substrate into sufficiently close contact with one another. It was shown by Nicloux that the lipase contained in oily seeds, such as those of the castor oil plant, is insoluble in water, but soluble in fatty media. The dried material obtained from the pancreas in many cases yields no lipase to water, but gives a strongly lipolytic solution when extracted with glycerol. The digestive function of bile, therefore, lies in its power of serving as a vehicle for the suspension and solution of the interacting fats, fatty acids, and fat-splitting enzyme. This vehicular function plays an important part in the absorption of fats and cholesterol. These pass through the striated basilar membrane bounding the intestinal side of the epithelium, either in a fine state of suspension (an emulsion), or dissolved in the bile in the form of fatty acids, or as soaps and glycerol (but see p. 953). On the arrival of these products of digestion in the epithelial cells, a process of resynthesis is set up. Droplets of neutral fat make their appearance in the cells, whence they are passed gradually into the central lacteal of the villus and so into the lymphatics of the mesentery and into the thoracic duct. The bile salts thus released from their function as carriers are absorbed by the blood circulating through the capillaries of the villi, and carried by the portal vein to the liver. On arrival they are once more taken up by the liver cells and turned out into the bile. Owing to the fact of their ready excretion by the liver cells, bile salts are the most reliable cholagogues with which we are acquainted. By this circulation of salts between liver and intestine, the synthetic work of the liver in the production of the bile salts is reduced to a minimum, and it has only to replace such of the bile salts as undergo destruction in the alimentary canal under the influence of micro-organisms, and are lost to the organism by passing out in the fæces as a gummy amorphous substance known as *dyslysin*. The action of pepsin is checked by bile owing to its inclusion in a precipitate which is thrown down.

JAUNDICE. If the bile ducts are ligatured, or obstructed in any way, so as to prevent the entry of bile into the intestine, the bile is reabsorbed and passes by the lymphatics into the blood stream. The bile pigment circulating in the blood is deposited in the various tissues of the body, giving them a yellow colour; bile pigments and bile salts are also excreted with the urine. This condition is known as jaundice. Certain types of jaundice have long been known in which no apparent obstruction of the bile ducts was present. It was therefore assumed that in these cases the bile pigment was set free in the blood, apart from the activity of the liver. Pathologists thus distinguished two kinds of jaundice, hepatogenous and hæmatogenous. The existence of the latter form was long disputed, although it was known that bilirubin could be formed as a product of the disintegration of hæmoglobin in old blood clots. F. C. Mann succeeded in totally extirpating the liver and, by means of injections of glucose, in keeping the animals (dogs) alive for thirty-six hours. He found that, six hours after the extirpation, bile pigment begins to accumulate in the blood and in the tissues, showing that this

substance can be formed as the result of the disintegration of hæmoglobin without the co-operation of the liver, thus justifying the long-disputed division of jaundice into the two varieties of hepatogenous and hæmatogenous.

This conversion of hæmoglobin into bilirubin occurs in the scattered intravascular macrophages in the liver, spleen and bone marrow, all of which belong to the reticulo-endothelial system.

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7. THE INTESTINAL JUICES

The juice of the small intestine consists of the secretion of Brunner's glands, and the *succus entericus*, or intestinal juice proper.

The glands of Brunner extend only over about an inch at the very beginning of the duodenum in the carnivora, but in the herbivora they may be found occupying the upper six inches of the intestine.

The secretion of Brunner's glands is rich in mucus, and is alkaline; it serves to protect the duodenal mucosa from the action of acid of the gastric contents. There is no secretion during fasting, but juice begins to be formed within an hour of the taking of food. Its formation is apparently due to an action of secretin and it occurs at normal rates after section of all nerves, and also in the transplanted duodenum.* The glands appear to contain a peptidase and an esterase.†

The intestinal juice is mainly secreted by the Lieberkühn's follicles. Besides its activating power on the pancreatic juice, the intestinal juice has numerous other functions to discharge in the digestion of the foodstuffs. Functionally there are many differences between the upper, middle and lower portions of the gut. Whereas the processes of secretion are better marked in the upper portions of the gut, absorption predominates in the lower sections, *i.e.* in the ileum. Much of the divergence in the statements which have been made with regard to the factors determining secretion and absorption in the small intestine is due to the failure to appreciate this difference between the activity of the mucous membrane at various levels.

The process of secretion in the small intestine may be studied by isolating loops, in an anæsthetised animal. Better results, however, may be obtained by establishing permanent fistulæ, the best type being the Thiry-Vella operation. This consists in cutting out a loop of intestine, and restoring the continuity of the gut by suturing the two ends from which the loop had been excised. Both ends of the excised loop are left open and brought into the abdominal wound. In such a fistula it is easy to introduce substances into the upper end and determine the flow of juice from the lower end, the constant emptying of the loop being provided for by the peristaltic contractions of its muscular coat. Intestinal fistulæ resulting from war injuries have been studied in man by Savitsch, and have given results confirming those made on dogs. Much can be learnt also by intestinal intubation in normal man.‡

* FLOREY and HARDING. *Proc. R. S.*, 1935, **117B**, 68; *Q. J. Exp. Phys.*, 1935, **25** 329.
† LINDERSTROM-LANG and HOLTER. *C. R. Lab. Carlsberg*, 1935, **20**, 42.
‡ OWLES. *Clin. Sci.*, 1937, **3**, 1.

In animals with intestinal fistulæ, a number of different conditions have been found to give rise to a flow of succus entericus, and so far no qualitative difference has been recorded between the upper and lower ends of the gut. A condition which will cause a free flow of juice from a fistula high up in the intestine will generally cause a slight flow from a fistula made from the ileum. Fasting animals show no secretion, or merely a scanty periodic flow about every two hours, from an empty isolated loop. Administration of food is also without regular effect on the isolated loop, so long as this remains empty. An important factor in exciting a flow of juice is local mechanical stimulation : thus the introduction of a drainage tube into the isolated loop is followed by a steady flow of succus entericus, whether the animal be fasting or fed. The flow of juice thus initiated is greatly modified by feeding the animal ; thus, a meal of fat will often double the rate of secretion. In most instances, however, the contents of the alimentary canal exert no influence on portions of the intestine with which they are not in contact.

It seems probable that the normal flow of intestinal juice is provoked by the presence, in the intestine, of its normal contents. One of the factors involved is the mechanical stimulus of the contents ; the other is probably a local chemical stimulation, which can be effected by various products of digestion.

The response to local mechanical or chemical excitation is apparently brought about through the local nervous mechanism of the intestine (Meissner's plexus), since section of the vagus does not abolish the reaction. The rate of the secretion can, however, be augmented by stimulation of the vagus, though only after a very long latent period (one hour), and the juice obtained by vagus excitation, unlike that resulting from local mechanical stimuli, is very rich in enzymes. (SAVITSCH.)

There are certain facts which seem to speak for an action of the central nervous system in the direction of inhibition of intestinal secretion. Thus it has been observed on many occasions that extirpation of the nerve plexuses of the abdomen, or section of the splanchnic nerve, causes a condition of diarrhœa which may last for two or three days. This condition might be determined either by an increased motor activity of the gut, or by removal of inhibitory impulses normally arriving at the intestinal glands. The latter view receives support from an experiment first performed by Moreau. The abdomen of a dog is opened under an anæsthetic, and three contiguous loops of small intestine are separated by means of ligatures from the rest of the gut. The middle loop is then denervated by destruction of all the nerve fibres lying on its blood vessels as they course through the mesentery, care being taken not to injure the blood vessels themselves. The loops are then replaced in the abdomen and the animal left from four to sixteen hours. On killing the animal at the end of this time it is often found that the middle loop, *i.e.* the denervated loop, is distended with fluid having all the properties of ordinary intestinal juice, whereas the other two loops are empty. A series of comparative experiments by Mendel and by Falloise have shown that the secretion begins about four hours after the operation, increases for about twelve hours, and then rapidly declines, so that at the end of two days all three loops will be found empty. It is possible, however, that the hyperæmia of the gut, which is produced by the process of denervation, may be sufficient to account for the increased formation of intestinal juice, since the hyperæmia will tend to pass off as the vessels recover a local tone.

Certain chemical substances introduced into the blood stream, *e.g.* histamine or pilocarpine, produce active secretion of the juice, and Nasset * has demonstrated the presence in the intestinal mucosa, of a secretory hormone, distinct from secretin, which excites the secretion of intestinal juice ; it is called *enterocrinin*. It is also possible that general humoral mechanisms may play a part, though probably only a subsidiary one, in bringing about normal secretion of succus entericus. Thus intravenous

* NASSET. *Amer. J. Physiol.*, 1938, **121** 481.

injection of secretin always causes a secretin of intestinal juice. This, however, may be entirely due to the presence of Nasset's specific enterocrinin.

A curious fact has been recorded by Savitsch, namely that the production of enterokinase depends upon the contact of the mucosa with pancreatic juice : in an isolated loop the enterokinase slowly disappears, but returns in a few minutes after introduction of pancreatic juice into the loop. The pancreatic juice thus in some way participates in the generation of its own activator.

Characters of Intestinal Juice. The intestinal juice obtained from a permanent fistula has a specific gravity of about 1010. It is generally turbid from the presence in it of migrated leucocytes, disintegrated epithelial cells and clumps of mucus. It contains about 1·5 per cent. total solids, of which 0·8 per cent. are inorganic and consist chiefly of sodium bicarbonate and sodium chloride. It is alkaline in reaction (pH = 8·3), but less buffered than pancreatic juice. The organic matter, besides a small amount of protein, includes a number of enzymes adapted to complete the processes of digestion commenced in the stomach and duodenum. It also contains the activator, *enterokinase*, which is necessary for the development of the full proteolytic powers of the pancreatic juice.

One of its most important enzymes * is known as *erepsin* ; it, or some similar enzyme, is present in almost all tissues of the body. It is distinguished by the fact that it has no power of acting on any native proteins, nor on peptones or proteoses, but only upon the polypeptides, which are rapidly split (optimal pH = 7·8). The enzyme is probably a mixture of several different peptidases which act on peptides of different structure, a dipeptidase acting on dipeptides, &c., and convert them into amino-acids—their ultimate cleavage products.

The succus entericus also contains the enzymes *lipase*, *protease*, *nuclease* and a *phosphatase*. Other enzymes of the intestinal juice are connected with the digestion of carbohydrates. In all mammals the intestinal juice is found to contain *invertase*, which transforms cane sugar into glucose and fructose, and *maltase*, which converts maltose into glucose. In young mammals the intestinal mucous membrane also contains *lactase*, which converts lactose into galactose and glucose. By these three enzymes, coming after the digestion of the starches by the amylases of the saliva and pancreatic juice, it is provided that all the carbohydrate food of the animal is transformed into hexoses.

The seat of origin of these various substances has been the subject of special investigations by Falloise. Whereas secretin can be obtained from the whole thickness of the mucous membrane, and is probably therefore contained in the epithelial cells covering the villi, as well as in those lining the follicles of Lieberkühn, a superficial scraping of the mucous membrane, which removes only the epithelial cells covering the villi, with the adherent mucous and intestinal secretion, gives a much more active solution of enterokinase than a deeper scraping. The most active solutions of enterokinase are, however, to be obtained from the fluid found in the cavity of the intestine after the injection of secretin. It seems possible that enterokinase is not present as such in the epithelial cells, but is first produced in the process of secretion and formation of the intestinal juice.

The enzymes, *erepsin*, *maltase*, *invertase* and *lactase*, probably pre-exist as such in the epithelial cells, especially in those lining the tubular glands of the gut, since mucous membrane pounded in water yields a solution of these enzymes which is generally more powerful in its action than the succus

* Cohnheim *Z. f. physiol. Chem.*, 1901, 33, 451.

entericus itself. The enzyme *arginase*, which splits up arginine into urea and ornithine, is only present in the cells. So great is the difference that many physiologists have suggested that the chief action of these enzymes occurs, not in the lumen of the gut, but in the passage of the foodstuffs through the epithelial cells of the small intestine on their way to the blood vessels.

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8. MOVEMENTS OF THE SMALL INTESTINE

The movements of the intestines can be investigated either by direct observation of the gut or by means of the barium meal and X-ray examination.* In using the former method it is essential to exclude the many reflex disturbing influences which can play on the intestine. These may be eliminated either by extirpation of the whole of the nerve plexuses of the abdomen, by division of the splanchnic nerves, or by destruction of the spinal cord below the mid-dorsal region. It is questionable, however, whether the movements seen under these conditions are the same as those which occur in the intact animal.

Three types of movement have been described in the small intestine, namely: (1) Segmenting movements; (2) peristalsis; (3) pendular movements. These three kinds of movements may be seen in an animal treated as described above, when its abdomen is opened in a bath of warm normal salt solution so as to exclude the disturbing influences of drying and cooling. The *pendular movements* are probably only seen in the opened abdomen, where they appear as side-to-side swaying movements of individual loops of the intestine. They are probably a consequence of a to-and-fro movement of intestinal contents resulting from intermittent contractions in different parts of the loops (ALVAREZ).

The *segmenting movements* are the most constant and fundamental. They may be recorded by inserting into the intestine a balloon filled with air and connected with a piston recorder. The constriction which accompanies each contraction is then seen to be most pronounced at the middle of the balloon, *i.e.* in the zone of greatest distension, and the amplitude of the contractions is augmented by increasing the tension on the walls of the gut. These movements are unaffected by the direct application of drugs, such as nicotine or cocaine, which we might expect to paralyse any local nervous structures in the intestinal wall; they occur equally well in the isolated intestine, from which facts Bayliss and Starling † concluded that these rhythmic contractions were myogenic, and were propagated from muscle fibre to muscle fibre. This is confirmed by the observation of Gunn and Underhill ‡ that the contractions continue in strips of muscle completely freed from any remains of the nerve plexus. These contractions occur with considerable regularity at a frequency which varies inversely with their distance from the stomach, and in the ileum is of the order of twelve a minute. The different frequencies at different levels is due, according to Alvarez, to the existence of a 'metabolic gradient' from above downwards, the upper portions having a higher

* CANNON. *Amer. J. Physiol.*, 1902, **5**, 257.
† BAYLISS and STARLING. *J. Physiol.*, 1901, **26**, 125.
‡ GUNN and UNDERHILL. *Quart. J. Exp. Phys.*, 1914, **8**, 275.

rate of metabolism and being also more excitable than the lower parts. These contractions must cause a thorough mixing of the contents of the gut with the digestive fluids. On examining under the X-rays the intestine of a cat, which has taken a large meal of bread and milk mixed with bismuth some hours previously, a length of gut may be seen in which the food

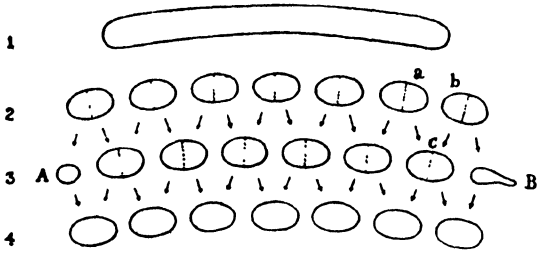

FIG. 553. Diagram of the 'Segmentation' Movements of the Intestines as observed by the Rontgen Rays, after Administration of Bismuth. (CANNON.)

1. A continuous column, intestinal movements being absent.
2. The column broken up into segments.
3. Five seconds later, each segment divided into two, the halves joining the corresponding halves of adjacent segments.
4. Condition (2) repeated five seconds later.

contents form a continuous column. Suddenly movements occur in this column, which is split into a number of equal segments. Within a few seconds each of these segments is halved, the corresponding halves of adjacent segments uniting. Again contractions recur in the original positions, dividing the newly formed segments of contents and re-forming the segments in the same position as at first (Fig. 553). In this way every particle of

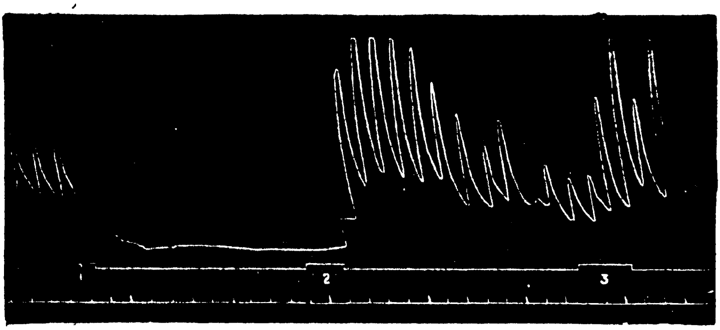

FIG. 554. Intestinal Contractions (Balloon Method).

In this dog all the abdominal ganglia had been excised, and both vagi cut. Showing propagated effects of mechanical stimulation above and below the balloon. (1) pinch above, (2) pinch below, (3) pinch below balloon.

food is brought successively into intimate contact with the intestinal wall. These movements have not a translatory effect, and a column of food may remain at the same level in the gut for a considerable time.

The *peristaltic* waves cause onward progress, possibly with a spiral rotation,* of the contents of the intestine. The classical description of peristalsis is that given by Bayliss and Starling, who described it as a wave moving slowly—generally at about 2 cms. a minute—involving contraction

* FRANKLIN and MAHER-LOUGHNAN. *J. Physiol.*, 1938, **94**, 426.

of the gut above the food mass, and relaxation below it. If a balloon be inserted in the lumen of the exposed gut, it will be found that pinching the gut *above* the balloon causes an immediate relaxation of the muscular wall in the neighbourhood of the balloon. On the other hand, pinching the gut half an inch below the situation of the balloon causes a strong continued contraction to occur at the level of the balloon itself (Fig. 554). Stimulation at any portion of the gut causes contraction above the point stimulated and relaxation below it (the 'myenteric reflex'). The same effect is produced by introduction of a bolus of food, especially if it be large or have a direct irritating effect on the wall of the gut (Fig. 555). In this case the contraction above and the inhibition below cause an onward movement of the bolus, which travels slowly down the whole length of the gut until it passes through the ileocæcal opening into the large intestine. According to Alvarez, the direction of the peristaltic wave is determined by the direction of the metabolic gradient of the intestine. The peristaltic contraction involves the co-operation of the local nervous system, viz.

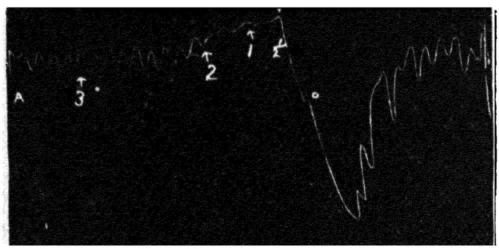

FIG. 555. Passage of Bolus. Contractions of Longitudinal Coat (Enterograph).

The bolus (of soap and cotton wool) was inserted into the intestine four inches above the recorded spot at A. The figures below the tracing indicate the distance of the middle of the bolus from the recording levers. As the bolus arrives two inches above the levers, there is cessation of the rhythmic contractions and inhibition of the tone of the muscle. This is followed, as the bolus is forced past, by a strong contraction in the rear of the bolus.

Auerbach's plexus. Whereas in the œsophagus it is the central nervous system which is involved, the peristaltic contractions in the small intestine continue after severance of all connection with the brain and spinal cord. On the other hand, they are absolutely abolished by painting the intestine with nicotine or with cocaine.

According to Alvarez, the peristaltic wave, as described by Bayliss and Starling, is abnormal, and in his opinion true peristalsis consists of a series of 'rushes' traversing the whole length of the small intestine, travelling much more rapidly (2 to 25 cm. per second), and in which the constriction is less intense and not preceded by a wave of relaxation.

Anti-peristalsis is never observed in the lower small intestine. Mall showed that, if a short length of gut be cut out and reinserted in the opposite direction, a species of partial obstruction results, in consequence of the fact that the peristaltic waves, started above the zone of operation, cannot travel downwards over the reversed length of gut. The intestine above this level therefore becomes dilated. If, however, the reactions of the local nervous system be paralysed or inhibited, a reflux of intestinal contents is quite possible, since the contractions excited at any spot by local stimulation of the muscle have the effect of driving the food either upwards or

downwards; the direction of movement of the food will be that of least resistance.

The movements of the small intestine are also affected by the central nervous system. Stimulation of the vagus causes an initial inhibition of the whole small intestine, followed by increased irritability and increased

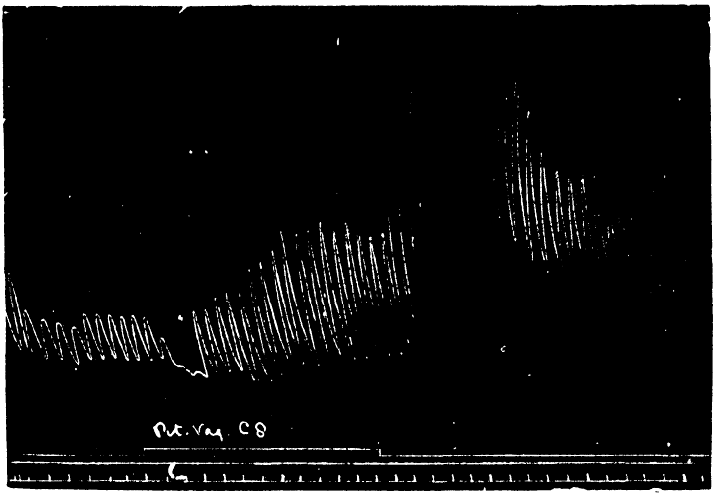

Fig. 556. Effect of Stimulation of Right Vagus on Intestinal Contractions.

contractions (Fig. 556). The ingestion of pleasant foods also increases intestinal tonus, probably by augmenting vagal impulses, while section of the vagus lowers tone. On the other hand, stimulation of the splanchnic nerves, or injection of adrenaline, causes complete relaxation of both coats of the small gut (Fig 557). It seems that the splanchnics normally exercise

Fig. 557. Excitation of both Splanchnic Nerves. Balloon Method. Intestine returned to Abdomen.

a tonic inhibitory influence on the intestinal movements, which can be increased reflexly by all manner of stimuli. On this account it is often impossible to obtain any movements in the exposed intestine so long as this remains in connection with the central nervous system through the splanchnic nerves. The relaxed condition of the gut, called paralytic ileus, which obtains in many abdominal affections, is probably also similar in origin, and is due to reflex inhibition through the splanchnic nerves.

The movements of the duodenum show some special features, viz. contraction of the first part (duodenal cap), peristalsis of the second and third parts, antiperistalsis of the second and third parts, and segmenting movements. Normally the contents are passed to and fro a few times in the duodenum before passing on to the jejunum, but once they have passed on they do not return. Antiperistalsis is most definite in the second part of the duodenum, and leads to periodic filling of the duodenal cap, or even to regurgitation into the stomach. Very rapid mixing of the contents with the digestive juices results from this to-and-fro movement.*

In man, the chyme remaining from the digestion of a meal begins to arrive at the end of the ileum in about three and a half hours after the meal. Here it accumulates for a while, during which time active segmentation movements occur, and further absorption of water takes place. Peristalsis is generally feeble in this part of the bowel, but is reflexly increased during or just after a meal. It is at this time, *i.e.* when the next meal is taken, that the contents are passed on by occasional peristaltic contractions through the lower end of the ileum and into the cæcum. The lowest 2 cm. of the ileum present a distinct thickening of the circular muscular coat, forming the *ileocolic sphincter.* This sphincter relaxes in front of a peristaltic wave and so allows the passage of food into the colon. On the other hand, it contracts as a rule against any regurgitation which might be caused by contractions in the colon.† Although thus falling into line with the rest of the muscular coat as concerns its reaction to stimuli arising in the gut above or below, it presents a marked contrast to the rest of the gut in its relation to the central nervous system. It is unaffected by stimulation of the vagus. Stimulation of the splanchnic, however, which causes complete relaxation of the lower part of the ileum with the rest of the small intestine, produces a strong contraction of the plain muscle forming the ileocolic sphincter (ELLIOTT).‡ According to Hurst,§ an important function of the ileocolic sphincter is, by its tonic contraction, to prevent the contents of the ileum passing too rapidly into the cæcum, so as to allow more time for digestion and absorption of foodstuffs to be carried to completion in the ileum.

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9. FUNCTIONS OF THE LARGE INTESTINE

Great differences are found in the size of the large intestine of different animals. In the carnivora it is short and narrow and possesses little or no cæcum. In herbivora the large intestine is well developed, with sacculated walls, and the cæcum is very large. Man occupies a somewhat intermediate position between these two classes. The great development of the large intestine in vegetable feeders is due to the fact that all the nutritious material in their food is enclosed in cells surrounded by cellulose walls. The cellulose is dissolved either by the agency of bacteria or by means of cytases in the vegetable cells themselves. In the horse and rabbit, for instance, the chief part of the digestion of the cellulose occurs by bacterial action in the cæcum.

In carnivora the large intestine has unimportant functions to dis-

* BOLTON and SALMOND. *Lancet*, 1927, 1230.
† RUTHERFORD. "The Ileo-cæcal Valve," 1914. (London: Lewis.)
‡ ELLIOTT. *J. Physiol.*, 1904, 31, 157.
§ HURST. *J. Physiol.*, 1913, 47 54.

charge in digestion and absorption. The products of digestion are practically entirely absorbed by the time that the food has arrived at the ileocolic valve. In man the importance of the large intestine will vary with the nature of his food. If, however, a large quantity of vegetable food be taken, a considerable amount of material may reach the large intestine unabsorbed. A small proportion of this may undergo absorption in the large intestine, while the rest will pass out with the fæces, increasing their bulk.

The large intestine in man is chiefly remarkable as the part of the alimentary canal in which micro-organisms flourish. In the mouth these are constantly present, including various species of *Actinomyces, spirochætes, spirilla, amœbæ*, &c.; the stomach has a very restricted flora in health, since the strong acid reaction of the contents kills most of the organisms which enter it. The upper small intestine also is often sterile, or at most contains a few acid-producing organisms; the ileum has a rapidly increasing bacterial content as the ileo-cæcal valve is approached. So far, however, there are few anaerobic bacteria and putrefaction is slight until near to the large intestine. In the large intestine itself an enormous and rapid growth of bacteria of various species occurs, *staphylococci, B. pyocyaneus, B. coli communis, B.B. perfringens, putrificus, sporogenes, butyricus* and many others. It has been estimated that up to 128,000 milliard bacteria per day are eliminated from the large intestine by the fæces; most of these are dead when passed.*

The study of the products of the activity of these bacteria, and of the protection of the body from these and from direct invasion by the organisms themselves, forms an interesting chapter in pathology.

The mucous membrane of the large intestine is lined by simple tubular glands containing many typical 'goblet' cells. Investigation by the fistula method shows that, when empty, the secretion of the large intestine is extremely scanty and composed wholly of mucus, while when stimulated mechanically by introduction of a drainage tube, a slight watery secretion is further contributed. The secretion in the cat's colon is effected through the action of the *nervi erigentes*.† Reflex secretion can be obtained by stimulating the central end of one nervus erigens, the other nerve being intact. Secretion is also caused by pilocarpine, inhibited by atropine, and reduced by sympathetic stimulation. The secretion has a pH of 8·4 and is rich in mucin, but almost devoid of any enzymes. In the resting state the colon reabsorbs the secretion. The secretion of mucus aids the passage of the fæces along the gut.

As an absorbing organ the large intestine of man is of little importance. From observations on cæcal fistulæ in man it has been calculated that about 500 c.c. of water pass the ileocolic valve in the twenty-four hours. Of this about 400 c.c., together with the water secreted in the large intestine itself, undergoes absorption in the large intestine. The absorption of food substances introduced into this part of the gut is much slower than that which takes place on introduction by the mouth. In some cases after the introduction of large enemata into the large intestine, a certain amount may escape backwards into the ileum and may there undergo absorption. The isolated large intestine of man is able to absorb only about 6 grammes of glucose per hour and about 80 c.c. of water. If egg albumin or caseinogen solutions be introduced by the rectum, no absorption can be detected after several hours. Feeding by nutrient enemata is thus of little value.

It was formerly held that the large intestine excreted a considerable fraction of the ingested calcium and magnesium salts, and also iron and heavy metals when these were administered. So far as calcium, magnesium and iron are concerned this belief has been shaken by the careful work of McCance

* LISBONNE. *Traité de Physiol.* (Roger & Binet), Vol. II. 1931, 445. (Paris: Masson.)
† WRIGHT, FLOREY and JENNINGS. *Q. J. Exp. Physiol.*, 1938, **28**, 207.

and Widdowson,* who found that when their salts were injected into the blood there was no increased output of the elements by the bowel. In earlier work allowance was not made for failure of absorption when these salts were given by mouth.

Such absorption of *iron* as takes place occurs in the duodenum and upper part of the jejunum. Only about 0·1 mg. daily appears in the urine; all the rest of the normal intake of about 7–8 mg. daily, together with any iron compounds given by mouth, is unabsorbed and appears in the fæces, chiefly as sulphide.

Of the acid radicals, *phosphates* may pass out either with the urine or with the fæces, the exact path taken being determined by the relative amount of calcium and alkali metals present in the food. If there is an excess of calcium most of the phosphates will leave with the fæces, but this does not imply that calcium phosphate has been excreted.

The large intestine does seem to be a channel of excretion for the heavy metals, such as bismuth and mercury. If bismuth be administered subcutaneously, the fæces will be found to contain this substance, and the wall of the large intestine will be stained black from a deposit of sulphide of bismuth. This deposit stops short at the ileocolic valve.

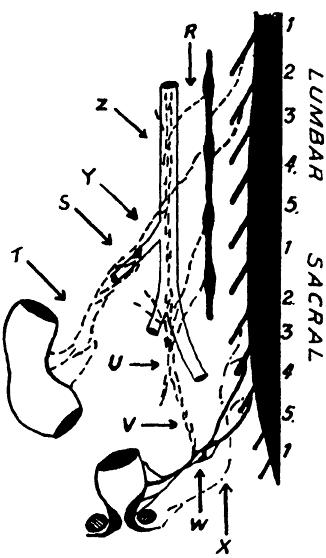

FIG. 558. The Nervous Outflow to the Distal Colon in Man. R., pelvic splanchnic nerves; S., inferior mesenteric plexus; T., inferior mesenteric nerves; U., presacral nerve; V., hypogastric nerve; W., hypogastric plexus; X., pudic nerve; Y., intermesenteric nerve; Z., intermesenteric plexus. (GARRY. *Physiol. Rev.*, 1934, **14**, 103.)

MOVEMENTS OF THE LARGE INTESTINE. By means of the occasional peristaltic contractions, accompanied by relaxation of the ileocolic sphincter, the contents of the small intestine are transferred into the colon. This happens most rapidly when the next meal is taken, but in any case not till about four and a half hours after the last one. When food is swallowed, or when chyme enters the duodenum, a peristaltic rush is initiated in the small intestine; on the arrival of the wave at the ileocæcal valve, the sphincter opens and allows the passage of some of the contents of the ileum into the colon ('gastro-ileal' reflex). In man the contents are considerable in bulk, are semi-fluid, and quickly fill up the transverse as well as the ascending colon.

Innervation. The innervation of the large intestine is derived from both sympathetic and parasympathetic sources; there are some differences in the details of anatomical distribution in its different parts, and in different species of animals. In man, the *colon* in its proximal part receives fibres from the superior mesenteric plexus; these are partly sympathetic, from the lumbar roots, and partly parasympathetic from the vagus. In its distal parts the colon is supplied by sympathetic fibres, which reach it from the upper lumbar roots *viâ* the pelvic splanchnic branches, intermesenteric plexus and nerves, the inferior mesenteric plexus, in which are many ganglia, and the inferior mesenteric nerves. The parasympathetic supply to the distal

* MCCANCE and WIDDOWSON. *J. Physiol.*, 1938, **94**, 148; *Bioch. J.*, 1939, **33**, 523.

colon is from the second to fourth sacral spinal roots, and then by way of the hypogastric plexus, in which are also scattered ganglia, to the wall of the colon (Fig. 558).

The rectum in man also receives a double nerve supply, of which the sympathetic is derived from the lumbar roots by way of the presacral nerve and the hypogastric nerves and plexus, while the parasympathetic supply from the sacral nerves also reaches it by way of the hypogastric plexus (Fig. 558). The *internal anal sphincter*, of plain muscle, has a similar supply, while the striated *external anal sphincter* receives a motor supply from the pudic nerve, which also supplies various other striated muscles of the perineum. Afferent fibres are present in the sacral and pudic nerves.

Although there are many discrepancies in the results of different investigators, we may take it as generally acceptable that the sympathetic supply exerts an inhibitory effect on the musculature of the colon, with the exception of the ileo-colic sphincter, to which it is motor, while the parasympathetic supply is motor to all except the ileo-colic and anal sphincters. Whether the vagus is motor to the cæcum and ascending colon has been disputed.

In addition to the nervous arrangements as described above, the large intestine also has two intrinsic nerve plexuses, similar to those of the small intestine.

Movements. The movements of the large intestine may be observed by the X-ray method assisted, in man, by auscultation and percussion. Until about four hours after breakfast the cæcum is filled with gas, is resonant on percussion, but emits no sounds. At about the fourth hour the ileal contents begin to enter, producing characteristic sounds, and the X-ray shadow shows rapid filling of the cæcum and ascending colon; these show little peristaltic action but seem to be passively filled by the peristaltic contractions of the ileum.

The movements of the large intestine differ considerably in various animals,* according to the nature of the food and the part played by this portion of the gut in the processes of absorption. Man takes an intermediate position between herbivora and carnivora as regards his large intestine.

In the cat there is active peristalsis and antiperistalsis of the ascending colon as it fills. The effect of the antiperistaltic waves is to force the food into the cæcum, regurgitation into the ileum being prevented by the tonic contraction of the ileocolic sphincter. The movements are rendered still more effective by the sacculation of the walls. The distension of the cæcum caused by this anti-peristalsis excites occasionally a true co-ordinated peristaltic wave which, starting in the cæcum, drives the food before it into the transverse colon. These waves die away before they reach the end of the colon, and the food is driven back again by waves of anti-peristalsis. The result of this process is the absorption of the greater part of the water of the intestinal contents, as well as of any nutrient material; and the drier part of the intestinal mass collects towards the splenic flexure, where it may be separated by transverse waves of constriction from the more fluid parts which are being driven to and fro in the proximal and intermediate portions. By means of occasional peristaltic waves these hard masses are driven into the distal part of the colon.

About 500 grammes of fluid chyme per day pass the ileocæcal valve in man; water and a little nutrient material is absorbed, chiefly in the cæcum and ascending colon, though the contents remain soft till they reach the

* ELLIOTT. *J. Physiol.*, 1904, **31**, 157; **32**, 401; ELLIOTT and BARCLAY-SMITH, *J. Physiol.*, 1904, **31**, 272

pelvic colon, when they undergo inspissation, so that the average weight of fæces per day is about 135 grammes.

The contents, in man, reach the hepatic flexure on an average in about six and a half hours after the meal, the splenic flexure at about nine hours, and the pelvic colon in twelve hours, though there are great individual variations. The time taken for a barium meal to reach various levels in the colon in man is shown in the accompanying figure (Fig. 559). It should be remembered, however, that a barium meal is usually given after previously emptying the whole alimentary canal as completely as possible. In the normal course of events food residues are much slower in being completely eliminated in the fæces than these figures would indicate. Thus Alvarez and Freedlander, administering coloured glass beads with the food to normal adults, found that, even after four days, only about three-quarters of the beads had been recovered.

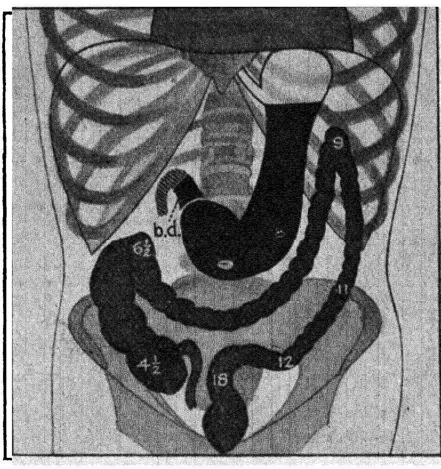

FIG. 559. Skiagram to show Normal Position of Colon in Man, and the Position attained by its Contents at different periods after a Meal containing Barium Sulphate.

The times of arrival at different levels are marked in hours after the meal on the colon. (HURST.)

The progress of the contents along the large intestine is not a slow regular one, but occurs as a result of a few rather sudden, powerful contractions. These, due to what is called a gastro-colic reflex, occur especially when food is taken, and during defæcation, and are called "*mass peristalsis.*" By this means the contents of the cæcum and ascending colon may be moved bodily into the transverse colon in a few minutes, and similarly the contents of the transverse colon may be equally rapidly passed on into the pelvic colon and rectum.

Defæcation. Under normal conditions, the rectum and distal colon are subordinated to the spinal and other nerve centres, the inhibitory effect of the sympathetic supply being predominant, and leading to retention of the contents. Hence spinal anæsthesia leads to some increase of tone, whereas spinal transection in the lower thoracic region leads to complete relaxation. It would seem that the sympathetic supply has its centre in the lumbar cord, while the parasympathetic centre is higher up.

The normal stimulus for defæcation is probably the attainment of a certain distension of the rectum, which is reinforced by stimuli arising from the movement of fæces along the rectum and anal canal. These reflexly excite contraction of the colon, and relaxation of the sphincters, the former being due to the transmission of impulses along the sacral nerves. Stimulation of these nerves in an animal produces a rapid shortening of the distal part of the colon, due to contraction of the recto-coccygeus and longitudinal fibres of the gut, followed after some seconds by a contraction of the circular coat. This originates certainly as high as the splenic flexure, and possibly even higher, and spreading rapidly downwards empties the whole of this segment of the gut. In man, as Hurst showed by the skiagraphic method, the pelvic colon becomes filled with fæces from below upwards, the rectum

remaining empty till just before defæcation. In individuals whose bowels are opened regularly, the entry of fæces into the rectum gives rise to a sensation of fulness and acts as the call to defæcation. 'Mass contractions' of the colon may occur as a reflex result of taking food into the stomach. The rectum thus becomes rapidly filled, and its distension produces the call to defæcation. Hence the act of defæcation after breakfast, or, in patients with irritable intestines, after any meal. If no response be made, the desire to defæcate passes away, since the rectum relaxes again. Hurst showed that the minimal pressure required to produce the call to defæcate varies from 30 to 40 mm. Hg. The emptying of the rectum itself is largely assisted by the contractions of the voluntary muscles of the abdominal walls and

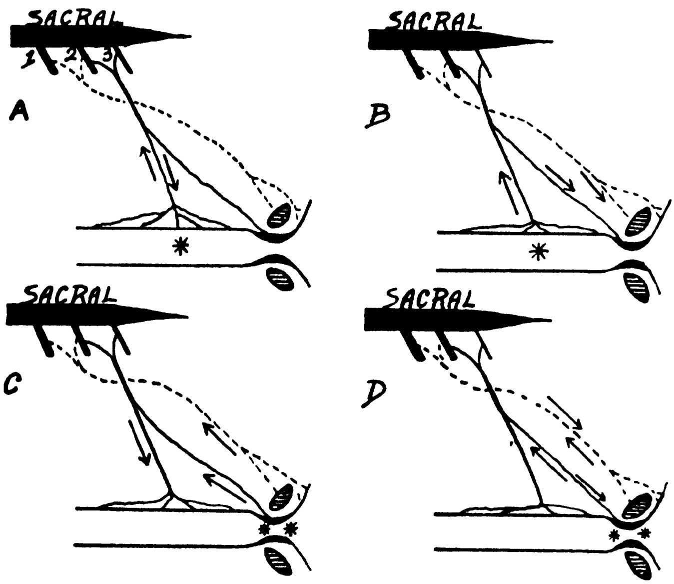

FIG. 560. Course of Impulses in Defæcation, according to Garry.
— pelvic nerve.
- - - pudic nerve.
* point of stimulation
Stimulus to mucosa of distal colon causes (A) contraction of distal colon and (B) relaxation of both anal sphincters.
Stimulus to mucosa of anus causes (C) contraction of the distal colon and (D) relaxation of both sphincters.

pelvic floor. The last section of the rectum is emptied at the close of the act by a forcible contraction of the levator ani and the other perinæal muscles, and this contraction also serves to restore the everted mucous membrane.

The anal orifice is guarded by an internal anal sphincter of smooth muscle, and by a series of three striated external sphincters.* The internal sphincter is supplied by the sacral and hypogastric nerves, and the external anal sphincters by the pudic nerve. These sphincters are normally kept closed, even on section of the upper lumbar spinal cord. Stimuli in the colon or rectum which give rise to defæcation cause a reciprocal contraction of the lower bowel and relaxation of these sphincters.† Conversely, stimulation of the anal canal, as by movement of fæces through it, leads to reflex dilatation of the canal, and contraction of the rectum. As shown in Fig. 560, the

* MILLIGAN and MORGAN. *Lancet*, 1934, **227**, 1150.
† GARRY, *J. Physiol.*, 1932, **74**, 14P; *Physiol. Rev.*, 1934, **14**, 103.

afferent nerves in these reflexes are the pelvic nerves and the pudic nerves, the former being also motor to the colon and inhibitory to the internal sphincter, while the latter is motor to the external anal sphincter.

The carrying out of this reflex act is normally dependent on the integrity of a certain part of the lumbar spinal cord. If this 'centre' be destroyed, the tonic contraction of the sphincter muscles disappears. The centre may either be excited to increased action, or be inhibited by peripheral stimulation of various nerves, or by emotions such as fear. Application of warmth to the region of the anus causes reflex relaxation of the sphincter; application of cold increases its tonic contraction.

Some time after destruction of the lumbar spinal centres a certain amount of automatic control is restored over defæcation. This has been supposed to be due to a fundamental control exerted by the local nerve plexuses,* but has not been finally explained.

THE FÆCES

In man or the carnivora the absorption of the constituents of a meal is practically complete by the time that the food has arrived at the lower end of the ileum. The fæces in the main are not derived from the food, but are produced almost entirely in the alimentary canal itself. This is shown by the fact that on analysing the fæces no soluble carbohydrates or proteins, albumoses, peptones or amino-acids are to be found. After a meal of meat, microscopic examination of the fæces reveals no trace of striated muscle fibres. Moreover, animals in a state of complete starvation form fæces which do not differ in their composition from the fæces which are found after feeding, though the amount is less. In one experiment Hermann isolated a loop of gut, joining its ends together so that a continuous ring was formed. The continuity of the gut was then restored by suturing the two free ends. After some weeks the isolated loop was found to contain a semi-solid material similar to fæces.

So long as vegetables or coarsely ground cereals are excluded from the diet, the nature of the latter does not alter the chemical constitution or appearance of the fæces. Under these circumstances the fæces have the following composition:

Water	65 to 67 per cent.
Nitrogen	5 to 9 ,,
Ether extract	12 to 18 ,,
Ash	11 to 22 ,,

The ash consists chiefly of lime and phosphoric acid with some iron and magnesia. The ethereal extract contains fatty acids and a small amount of lecithin. Neutral fat is present in very small proportions. The fæces also contain small quantities of cholic acid and its decomposition product, dyslysin, also coprosterol, a reduction product of cholesterol, and a certain amount of purine bases, consisting of guanine, adenine, xanthine and hypoxanthine. On the average the fæces contain about 0·11 gramme of purine bases per diem, about seven times as much as is contained in the urine passed in the same time. The material basis of the fæces seems to be largely inspissated mucus, bile and other secretions, desquamated epithelial cells from the intestinal wall, and bacteria, of which countless numbers, chiefly dead, are present. It has been reckoned that as much as 50 per cent. by weight of the fæces may consist of dead bacteria.

Very different is the composition of fæces if the food contains a large

* DENNY-BROWN and ROBERTSON. *Brain*, 1935, **58**, 256.

amount of cellulose. Not only does the ingested cellulose pass unchanged into the fæces, but large quantities of other substances enclosed in the cellulose walls may also escape digestion. Moreover the increased bulk of the undigested residue stimulates peristalsis, and thus quickens the passage of the food through the gut. The influence of the character of the food is well illustrated by a comparison of the amount and composition of the fæces on different kinds of bread (RUBNER):

Kind of bread.	Weight of moist fæces.	Weight of dried fæces.	Percentage of ingested food	Nitrogen (grms.).
Bread from fine flour	132·7	24·8	4·03	2·17
Bread from coarse flour	252·8	40·8	6·66	3·24
Brown bread	317·8	75·79	12·23	3·80

The indigestible cellulose in the food is not without value. If the food is capable of entire digestion and absorption, the amount of fæces formed is limited to that produced by the intestinal wall itself. The small bulk exercises very little stimulating effect on the intestine, and the movements of the latter will therefore tend to be sluggish. The presence of a certain amount of cellulose in the diet may therefore be of considerable advantage by giving bulk to the fæces and ensuring the proper regular evacuation of the lower gut.

10. The Sensations of Hunger and Thirst. When the body is in need of food or water, the characteristic sensations of hunger and thirst arise and increase in intensity if the need is not satisfied. It is important to distinguish between hunger proper and appetite: the former is unpleasant or even painful, while the latter, though accentuated by actual hunger, is mainly psychological and generally pleasurable.

It is not certain what is the fundamental cause of the hunger sensations. Hunger may be specific, as well as general, as, for example, the characteristic salt-hunger which leads animals in the wild state to travel long distances in search of its relief. Indeed, the constant average dietary balance in man and in animals of all species must in the long run be due, not so much to any conscious selection of the various constituents as to an unconscious choice guided by vague specific desires, now for more carbohydrate, now for salt, or fat, or protein. We have no knowledge of the mechanisms upon which these specific hungers, or their satisfaction, depend, nor could it be denied with any certainty that general hunger is due to a summation of such specific hungers, though probably it is not. The view that general hunger is associated with a lowering of the blood sugar receives some support from the fact that the blood sugar is a common pabulum into which certain other nutritional materials are convertible, so that its concentration in the blood may well serve as an index of the nutritional needs of the body. In support of this view is the fact that a sensation of hunger is one of the first effects of lowered blood sugar resulting from the action of a dose of insulin, though against it is the fact that hunger is a very prominent symptom in diabetics in whom, however, the blood sugar is permanently raised.

As regards the actual pangs of hunger, which are referred to the epigastric region, there is little doubt that these are caused by powerful contractions of the body of the stomach, which begin as soon as the organ is empty or nearly empty,* at first as feeble contractions at intervals of twenty to thirty

* CARLSON, "Control of Hunger in Health and Disease." Chicago, 1916.

seconds, but finally becoming very powerful and lasting for about thirty seconds. Each contraction is accompanied by a hunger pang, and by an increased secretion of saliva.

The effects of deprivation of water are evident sooner than those of want of solid food, and are correspondingly more urgent. In the first stage there is craving for liquid, and a feeling of dryness in the mouth ; later the dryness increases, the air breathed seems hot and dry, and frequent and painful swallowing movements are made ; food cannot be swallowed and even sugar may not dissolve in the mouth. Delirium and death soon follow, the total duration of life without water depending on the rate of loss and so being brief (one to two days) after exertion in hot dry climates, but longer, up to two weeks or more, in cooler damper climates and under conditions of rest.

The thirst of *diabetes mellitus* and of *diabetes insipidus* is imperative : in the former case the water is lost in company with the sugar excreted, in the latter there is a primary disturbance of the normal water distribution of the body, so that water which should normally be stored, *e.g.* in the connective tissues and liver, is excreted and leads to a constant shortage.

It has often been supposed that thirst is a general sensation arising in some way from the desiccated tissues. This view received support from experiments by Claude Bernard, who found that in animals with double œsophagostomy, as for sham feeding, or animals with open gastric fistulæ through which fluid escaped on swallowing, thirst was apparently not relieved by drinking. It has certainly been found that after long deprivation of water the osmotic pressure of the blood is demonstrably increased,* and possibly this is the case in diabetes mellitus, yet it is not possible to show any clear change in that direction in the early stages, in which, nevertheless, thirst is well established. Probably, therefore, the changes in the blood are too small to detect by physico-chemical methods, though sufficient to produce the characteristic sensations of thirst.

The alternative view, supported recently by Cannon,† is that the sensation of thirst is, as it appears to be, due to a dryness of the mouth, from any cause. Owing to the passage of the inspired air through the pharynx, this always tends to become dried, but drying is ordinarily prevented by the secretion of saliva. When the body is deprived of water, however, the secretion of saliva is reduced, thus leading to the slight drying of the pharynx and producing the sensation of thirst. Administration of atropine, by checking the secretion of saliva, gives rise to thirst, and conversely pilocarpine when administered to thirsting animals relieves it (Pack.)‡

It has been stated also that after ligature of the parotid ducts, dogs show constant thirst. But doubt has been cast on this by Montgomery,§ who finds that removal of all the salivary glands from dogs does not alter the water intake. The same is true of men with no salivary glands ; they drink no more than others, but drink more often.||

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* Mayer. *C. R. Soc. Biol.*, 1900, **52**, 153.
† Cannon. *Proc. Roy. Soc.*, 1918, **90B**, 283.
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CHAPTER XLV

THE ABSORPTION OF THE FOODSTUFFS

THE processes of digestion result in the conversion of the insoluble constituents of the food into soluble substances of smaller molecular weight, which can pass into the blood stream, and so become available for use in the body. The transfer of the dissolved materials, together with water, from the intestine into the body fluids, is called absorption.

Structure of Villi. The absorption of water and saline fluids is

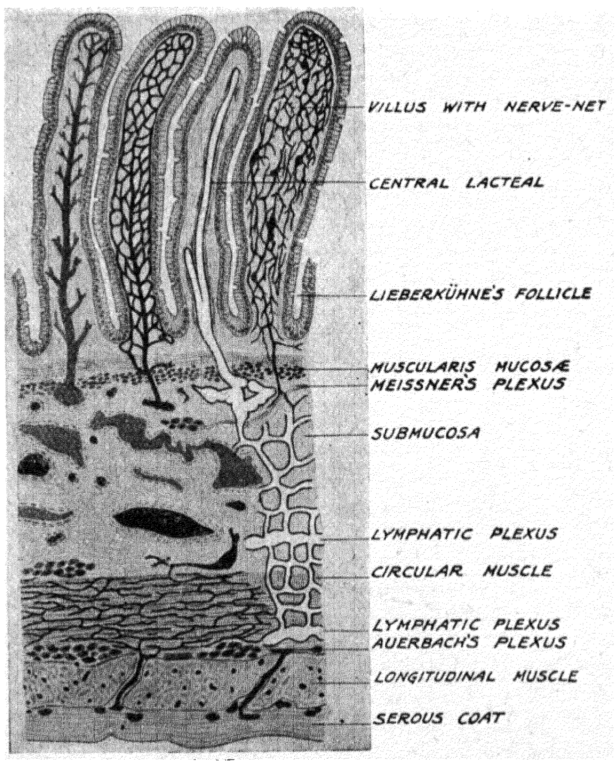

FIG. 561. Diagrammatic Section through Wall of small Intestine to show Vascular and Lymphatic Arrangements. (After SCHAFFER.) (*Lehrb. d. Histol. u. Histogenese*, 1922.)

greatly facilitated by the presence of the villi of the small intestine. By means of these structures the absorbing surface of the intestine is largely increased. It has been calculated that each square millimetre of intestine represents an absorbing surface of 3 to 18 mm.^{2}, and a total capillary surface of about the same order, though not all these capillary surfaces are superficial in position. Each villus (Fig. 561) consists of a framework of reticular tissue containing many leucocytes in its meshes, separated from the lumen of the gut by a continuous layer of columnar cells. These surface cells, which

rest on an incomplete basement membrane, are of four kinds. Much the most abundant are the *principal* or *columnar absorbing cells*. These present a striated border or fringe on their outer sides (Fig. 564). Scattered among them are numerous *goblet cells*, *Paneth cells* and *enterochrome* or *basal granular cells*.* Similar groups of cells are found lining the crypts of Lieberkühn. Apart from the fact that the goblet cells secrete mucus, the function of these various cells is a matter for speculation.

The villus offers two channels by means of which material which has passed through its epithelium may be carried away. In the middle of the villus is the central lacteal, a lymph capillary closed at its extremity and bounded by a complete layer of delicate endothelial cells. This leads into a plexus of lymphatics placed superficially to the *muscularis mucosæ*. From the superficial plexus communicating branches pass vertically to a corresponding plexus lying in the submucosa. Valves are present in abundance in the deeper plexus, so that fluid can pass easily from the lacteal to the deeper plexus, but not in the reverse direction. From the muscularis mucosæ, unstriated muscle fibres pass up through the villus, to be attached partly to the outer surface of the central lacteal, partly by expanded extremities to the basement membrane covering the surface of the villus. Contraction of these muscle fibres will tend to empty the central lacteal into the deep plexus of lymphatics and may also cause an expulsion of the contents of the spaces of the retiform tissue of the villus into the central lacteal, so that any of the intestinal contents which have made their way into the spaces of the villus might be forced into this lymph channel. The alimentary canal represents one of the few localities where a formation of lymph is constantly proceeding, even in a condition of complete rest. On placing a cannula in the thoracic duct of a dog, an outflow of lymph is obtained which may vary in different animals between 1 c.c. and 10 c.c. in ten minutes. The greater part of this lymph is derived from the alimentary canal.

The other possible channel of absorption is by the capillary blood vessels of the villus. Each villus is supplied with blood from one or two arterioles which break up into a rich plexus of capillaries lying close under the basement membrane of the villus. The total surface area of the portions of the outer capillary walls which lie immediately below the epithelium is about one-third of the whole epithelial surface, so that the surface through which interchange may occur between the contents of the bowel and the blood is roughly three to five times the area of the intestinal mucosa in plan. In these capillaries the blood is circulating rapidly, so that a considerable amount of material may pass into them from the spaces of the villus without appreciably altering the percentage composition of the blood. But it must be remembered that the blood in these vessels is at a high pressure, probably not less than 30 mm. Hg, so that we shall have to place out of court any possibility of the absorption occurring in consequence of hydrostatic differences of pressure, *i.e.* by a process of filtration. The return blood is collected into one or two veins, which run to a submucous plexus and then join the radicles of the portal vein in the mesentery.

Movements of Villi. One of the most striking facts in connection with intestinal absorption is that when it is in progress the villi are in a state of continual movement,† which can easily be seen on opening the intestine opposite the mesenteric border in a chloralosed dog and examining

* MACKLIN and MACKLIN. In "Cowdray's Special Cytology," 1935 p. 233.

† HAMBLETON. *Amer. J. Physiol.*, 1914, **34**, 446; KING and ARNOLD. *Am. J. Physiol.*, 1922, **59**, 97; KING, ARNOLD and CHURCH. *ibid.*, **61**, 80; KOKAS. *Pflüger's Arch.*, 1930, **225**, 416, 421; 1932, **229**, 486; VERZÁR. *Ergebn. d. Physiol.*, 1931 **32**, 391; KOKAS and LUDÁNY, *Pflüger's Arch.*, 1932, **231**, 20 (isolated intestine).

the mucosa by reflected light with a binocular microscope (Fig. 562). The movement in carnivora consists of a shortening, at rather irregular intervals of time (about six per minute) to about half the extended length, and then a more gradual extension; though the villus on contraction becomes shorter, it does not get much thicker, so that it expels some of its contents, probably its lymph. The cause of the shortening is no doubt the contraction of the plain muscle of the villus, but the lengthening has not been fully explained. The villi will move quite independently of one another. In herbivorous and omnivorous animals the movements are somewhat different.

In the fasting state the villi are motionless and flattened so as to look elliptical when seen from above, but if moistened with water or Ringer's solution they immediately swell out to a rounded cross-section and at once begin their pumping movements. The movements usually cease soon after removal of the intestine from the body, after loss of blood, stoppage of circulation, asphyxia or acapnia.

Mechanical stimuli to the base of the villus cause a contraction, probably by exciting the Meissner's plexus, which appears to be motor to the plain muscle of the villi. Chemical stimuli, *e.g.* physostigmine, yeast extract, amino-acids, &c., also cause rhythmic contractions of the villi. When the

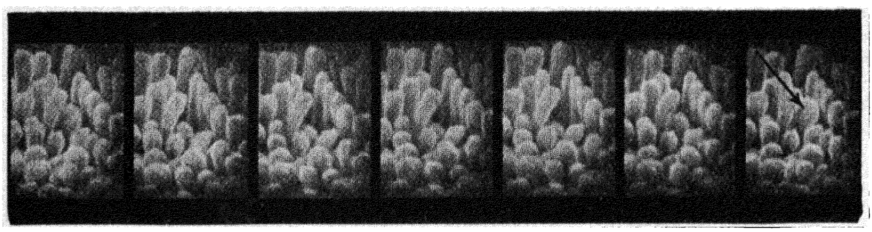

FIG. 562. Portion of cinematomicrograph film showing movements of villi in living intestine of dog during somewhat less than one second. Note the villus indicated by the arrow. (KOKAS and LUDÁNY, *Pflüger's Arch.*, 1930, **225**, 421.)

mucosa is stripped off from the submucosa these stimuli all fail if the muscularis mucosæ, together with Meissner's plexus, is detached, but remain effective when it is left behind. The natural stimulus for the contractions of the villi is stated to be a hormone, called *villikinin*. It is extractable from the intestinal mucosa by dilute hydrochloric acid and has been shown, by cross-circulation experiments, to be present in the blood after hydrochloric acid has been placed in the duodenum of a donor dog.*

THE ABSORPTION OF WATER AND DISSOLVED SUBSTANCES. The absorption of water from the alimentary canal is very little influenced by the water content of the body. It is practically impossible, however large the quantities of fluid ingested, to evoke the production of fluid motions, the greater part of the ingested fluid being absorbed in the alimentary canal. Thus a man may keep himself in perfect health and maintain the water balance of his body steady, whether he take one litre or six litres of water daily. The whole process of regulation, apart from that determined by appetite, is carried out at the other end of the cycle, viz. by the kidneys.

The absorption of water in the stomach may be regarded as nil. Although from this viscus alcohol and some dissolved substances, *e.g.*, sugar, may be absorbed to a slight extent, water or saline fluids introduced into it

* KOKAS and LUDÁNY. *Q. J. Exp. Physiol.*, 1938 **28**, 15.

are passed through the pylorus, either without change, or increased by the secretion of gastric juice. The chief absorption of water occurs in the small intestine. It is on this account that the salient features of cases of dilatation of the stomach with stenosis of the pyloric orifice can be nearly all referred to the starvation of the body in water, and can be often relieved by the administration of water, either subcutaneously or by the rectum, *i.e.* by the channels through which absorption is still possible. Water that has been swallowed to quench thirst has first to be passed from the stomach into the small intestine before it can be absorbed and relieve the needs of the tissues. The intestinal contents at the ileocæcal valve contain relatively nearly as much water as they do at the upper part of the jejunum. Their absolute bulk, however, is much smaller, so that only a small proportion of the water that has been taken in by the mouth remains to be absorbed in the large gut—an amount probably much less than that which has been added to the contents of the small intestine in the form of secretions.

When salt solutions are introduced into the small intestine, they are rapidly absorbed without the production of any corresponding increase in the rate of lymph flow from the thoracic duct. On the other hand, the absorption of large amounts of fluid may cause a diminution of the solids of the plasma. This has usually been interpreted as meaning that the capillary network of blood vessels at the surface of the villi is solely responsible for the absorption. But an alternative explanation is possible. When the villi contract they expel lymph : on its way through the sub-mucosa the lymph vessel comes into very close contact with special plexuses of small veins, so that it would be quite possible for reabsorption of water or dissolved material of the lymph to take place into the blood. Hence, in addition to absorbing from the intestinal contents into the capillaries of the villi, there might also be reabsorption from lymph into blood, of various materials of the lymph.

The problem as to the forces which are responsible for the transfer of various substances from the lumen of the intestine across the 'membrane' consisting of epithelial cells, together with interstitial tissue and endothelium of blood or lymph capillary, into blood or lymph respectively, is a very old one to physiology, and still presents many difficulties. As regards the *rate* at which the transport occurs, it must be granted that this will be largely influenced by the contractile activity of the villi referred to above. Setting this aside, however, as likely to affect only the quantitative aspect in certain respects, the problem still allows of no facile solution by reference to simple laws of physical chemistry, probably because our acquaintance with those laws is not yet sufficiently complete, and as it becomes more so they assume a more complex form. Opinions, therefore, run in two directions ; according to one view the living intestinal membrane selects those constituents which are to be absorbed, in accordance with the needs of the organism ; the other school considers that though the process of absorption may be vastly complicated by special physico-chemical properties of the membrane, and by the incidence of various modifying influences, yet it is not at present desirable to regard it as fundamentally different from that concerned in the passage of substances through dead membranes. Some of the factors concerned in transport through membranes in general have already been discussed in Chapter VII.

The special physico-chemical phenomena which need some further comment here may be grouped under the headings : (1) Diffusion and Osmosis ; (2) The Special Properties of the Membrane ; (3) Hydrotropy.

(1) Diffusion and Osmosis. These terms have already been explained in Chapter VII., and the reader is here only reminded of some pertinent facts which are in apparent contradiction to the accepted laws of osmosis and diffusion. As an example we may take some experiments * on the passage of water and sodium chloride from the intestine of dogs with Thiry-Vella fistulæ, during half-hour periods, after placing 75 c.c. of solution in the loop. When distilled water was placed in the gut, only 59 per cent. of it was absorbed, but when 0·4 per cent. NaCl was used, as much as 95 per cent. of the water was absorbed. With ascending strengths of NaCl solution, the water absorbed rose from 59 per cent. with pure water to a maximum of 95 per cent. between 0·4 and 0·7 per cent. NaCl, and then fell rapidly because with concentrations over 1·8 per cent. osmotic passage of fluid *into* the intestine, as well as considerable secretion, took place. The absolute amount of sodium chloride absorbed increased with the concentration of NaCl placed in the intestine, up to 1·5 per cent., *beyond which it fell*; but the percentage of the NaCl given which was removed rose with concentration until about 0·7 per cent. and then fell. At 0·1—1·2 per cent. NaCl (when allowance for secretion into the gut was made) the fluid was absorbed without change of composition, *i.e.* water and NaCl were absorbed together; from solutions over 1·2 per cent. NaCl, however, the chloride was absorbed faster than the water. Some of these facts might be explicable on the simple laws of osmosis, but some certainly cannot be so explained. The absorption of isotonic solutions has been supposed to be accounted for by the osmotic pressure of the colloids in the blood plasma or lymph within the spaces of the villi, but Rabinovitch found that the rate of absorption of both the salt and water from 0·9 per cent. NaCl solution was not altered by the addition to it of 6 per cent. gum acacia, which provides a colloid osmotic pressure about equal to that of the plasma.

When isotonic solutions of sodium chloride and sodium sulphate are placed in an intestinal loop the sodium chloride is almost completely absorbed, although blood plasma contains at least 0·6 per cent. of that salt, whereas the sodium sulphate is unabsorbed and remains in approximately isotonic solution. Similarly, magnesium ions are unabsorbed.† It is clear, therefore, that osmotic work is performed by the intestine even in an apparently simple case like this one, when poisoned by cyanide, the difference between the intestinal mucosa and a dead membrane disappeared.

An argument in favour of active intervention of the cells of the gut in absorption is perhaps furnished by the fact that if an animal's own serum be introduced into a loop of its intestine the whole of the serum is absorbed. It is impossible to explain this result as due to the digestion of the proteins and their conversion into diffusible products, since the intestinal loops were washed free of any trypsin, and serum has a strong antitryptic action which would prevent its being attacked by traces of trypsin. It has also been claimed by Mills ‡ that tissue fibrinogens can be absorbed from the intestine and recovered unchanged from the urine.

Wallace and Cushny,§ as the result of their experiments on the relative absorbability of various sodium salt solutions from the gut, divide the salts into four main classes as follows: (1) Sodium chloride, bromide, iodide, formate, acetate, propionate, butyrate, valerianate, caprate; (2) Ethyl sulphate, nitrate, lactate, salicylate, phthalate; (3) Sulphate, phosphate, ferrocyanide, caprylate, malonate, succinate, malate, citrate, tartrate; (4) Oxalate, fluoride.

* Rabinovitch. *Amer. Journ. Physiol.*, 1927, **82**, 279.

† Ingraham and Visscher. *Amer. J. Physiol.*, 1938, **121**, 771; Ingraham, Peters and Visscher. *J. Physical Chem.*, 1938, **42**, 141.

‡ Mills. *Amer. J. Physiol.*, 1923, **63**, 484.

§ Wallace and Cushny. *Amer. J. Physiol.*, 1898, **1**, 411; *Pflüger's Arch.*, 1899, **77**, 202.

Of these the first class contains salts which are absorbed with great ease from the intestine. The second are absorbed with somewhat greater difficulty. The third group are absorbed so slowly, *i.e.* the salts retain the water in which they are dissolved so long, that they act as laxatives. The members of the fourth class are not absorbed at all. It is evident that this classification is independent of the diffusibility of the salts. There is, however, one character which apparently determines the non-absorbability (relative or absolute) of the members of the third and fourth classes. All these salts form insoluble compounds with calcium. This common character is not an explanation of the permeability of the cell wall, but is simply a general statement of one of the conditions which affect the power of the cells to take up salts from their solutions, this power being absent in the case of salts which furnish an insoluble calcium compound.

The active intervention of the cells in the absorption of salt solutions and serum can be abolished by any means which diminishes or destroys their vitality, such as the addition of sodium fluoride to the fluid to be absorbed, or destruction of the epithelium by previous temporary occlusion of the blood vessels supplying the loop of intestine.

When sugar solutions are used, both sugar and water are completely absorbed, but again certain anomalies appear, which are not readily explained on physico-chemical grounds. Glucose disappears almost as rapidly as sodium chloride or iodide, though its physical diffusibility is less than that of these salts. Further, great differences, which are also not referable to the diffusibility of the sugars in question, are found between the rates at which different sugars are absorbed and in different species of animals.* In cats the order of speeds of absorption is galactose $>$ xylose $=$ glucose $>$ fructose. In rats, galactose is the most rapidly absorbed, then comes glucose, while fructose is only half, and mannose only one-fifth as rapidly absorbed as glucose,† the actual ratios of rates being 110, 100, 43 and 19 respectively. The pentoses xylose and arabinose are very slowly absorbed (15 and 9 respectively); when the intestine is dead, and used like a dialyser, the rates are different, for then, though all substances pass more quickly, xylose passes out even more rapidly than glucose.‡ There is never any passage of glucose outwards from the blood into the lumen of the bowel.

When the rate of absorption of glucose from solutions of different strengths is studied, it is found that the most rapid absorption is from 0·75M (13·5 per cent.) solution, the rate of absorption from this strength being about 20 per cent. more rapid than from 2M solutions.§ A still greater rate of absorption was obtained when 0·2 per cent. phosphate was added to the 0·75M glucose solution. There is considerable evidence that glucose, and probably other sugars, are absorbed in the form of combinations with phosphoric acid. Thus, serum phosphate is increased during absorption,‖ while if the phosphatase be poisoned, *e.g.* by phlorrhizin, absorption of glucose from the intestine is inhibited.¶

As regards the disaccharides, it would seem that unless these are hydrolysed by intestinal enzymes there is no absorption at all, though they are diffusible readily enough through dead membranes.

It is a further curious fact that some substances, *e.g.* glucose, or amino-acids, are absorbed at a constant rate from the intestine, in spite of the fact that as absorption proceeds the concentration of the substance in the gut diminishes, yet other substances, such as lactic acid and iodides, are

* DAVIDSON and GARREY. *J. Physiol.*, 1940, **97**, 509.
† CORI. *Physiol. Rev.*, 1931, **11**, 145.
‡ AUCHINACHIE, MACLEOD and MAGEE. *J. Physiol.*, 1930, **69**, 185.
§ MAGEE and REID. *Journ. Physiol.*, 1931, **73**, 163.
‖ BODANSKY. *J. Biol. Chem.*, 1934, **104**, 473.
¶ LUNDSGAARD. *Biochem. Ztschr.*, 1933, **264**, 221.

absorbed at rates which depend on the concentration of the substance in the intestine at the time.*

When we turn from these facts to seek physico-chemical explanations in accordance with the laws of osmosis, it is necessary, if we are to make any further progress, to consider the latter in a wider sense than has hitherto been customary in physiology. In the narrower sense, osmosis refers only to the passage of water through membranes impermeable to all solutes, but it is clear that we must consider the laws of the passage of both water and solutes through membranes of restricted, but variable, permeability. These laws are complex, and have been specially studied by Schreinemakers,† who finds that the course of the interchange of water and solutes will vary, and in an unpredictable manner, according to the number of solutes, their concentration, and the nature of the membrane, so that it is quite possible, for instance, even with dead membranes, to get water or solute moving in a direction which is apparently opposed to simple laws of osmosis as deduced from semi-permeable membranes. It would be beyond our scope here to enter further into this aspect of the subject.

We must conclude that, when a fluid is introduced into the intestine, an active transference of water and solutes from the lumen into the blood stream is effected by the intermediation of forces having their origin partly in the metabolism of the cells themselves. This work of absorption of the cells may be aided or hindered according to the physical conditions present, though our knowledge of these physical conditions is at present too fragmentary to enable us to appraise the respective parts played, on the one hand by the physical conditions and on the other hand by the activity of the cells, which primarily results from and secondarily modifies them by introducing fresh ones. If these act against the cells, *e.g.* if the fluid be hypertonic, the absorption is effected more slowly, while with hypotonic solutions the physical conditions concur with the vital activity of the cells in bringing about a very rapid transference of fluid from the gut into the blood vessels. Among these physical conditions we must reckon the nature of the salts present in the solution. If these can pass easily into, and through, the cells, *e.g.* ammonium salts, sodium chloride, absorption is carried out rapidly. If, on the other hand, the salts in the intestinal contents are but slightly diffusible, or have very little power of penetrating into the cells, the absorption of water by the cells causes an increased concentration of the salts, and therefore an increased osmotic pressure which offers a resistance to any further absorption.

(2) Special Properties of the Membrane. As already explained in Chapter VII., the results of osmotic experiments, even with dead membranes, depend very much on the properties of the membrane, and this in its turn is dependent, not only on its chemical composition, but, since the membranes with which we are concerned are essentially colloidal, also on its previous history.

Like other cells, those of the intestinal epithelium are bounded on their free surface by a 'lipide' layer, permeable only by such substances as are soluble in lipides. On the other hand, the cement substance between the cells may be of a different character and possibly permeable to water-soluble substances. The question has been propounded whether the greater part of the substances which enter the blood plasma from the gut pass between the cells or through them. Water could probably pass in either way. Most of the inorganic salts, as well as the very important constituents of the food, the sugars, are insoluble in lipoids and would have to pass between the cells. When the question is investigated by the use of dyestuffs, soluble or insoluble in lipides, it is found that the lipide-soluble dyestuffs, such as neutral red or toluidin blue, pass into the cells, whereas the dyestuffs which are insoluble in such substances pass into the intercellular spaces. Too much stress, however, must not be laid on these experiments. The fact

* Cori and Cori. *J. Biol. Chem.*, 1928, **81**, 389.

† Schreinemakers. *J. Gen. Physiol.*, 1928, **11**, 701; 1929, **12**, 555; 1930, **13**, 335.

that absorption of these dyestuffs is determined by the physical conditions of the cell membrane is no proof that the absorption of the normal food constituents is determined in the same way. In fact, it is quite legitimate to assume that the lipide membrane or limiting layer round every cell has as its main office, not the regulation of the access of foodstuffs to the cell, but its protection from anything which it does not require. If it were not for such a membrane the assimilation of a salt would be determined entirely by its concentration in the immediate surroundings of the cell, whereas we know that assimilation by any living organism, whether uni- or multi-cellular, is regulated in the first place by the activity of the organism itself. The properties of the membranes are also liable to be altered by such phenomena as adsorption, electric charges, imbibition, Donnan equilibria, &c.

(3) Hydrotropy. Hydrotropy * is the property of certain substances to render other substances soluble in water, in which otherwise they would be insoluble. Hydrotropic substances are usually salts of certain organic acids which powerfully lower surface tension, and many examples are now known of synthetic as well as of naturally occurring hydrotropic substances; the substances which are brought into solution by their agency are numerous, and include fats, lipides, sterols and certain inorganic salts, *e.g.* calcium carbonate and magnesium phosphate. The increase of solubility effected by the agency of hydrotropic substances is sometimes remarkable, as, for instance, a 100-fold increase in the solubility of sulphonal by sodium salicylate, a five-fold increase for uric acid by atophan, &c.

The hydrotropic substances of the body have been studied by Verzár and Kúthy,† who have found such substances, of unknown nature, present in many tissues, but especially abundant in the intestine, and in blood. They have shown that various substances, but particularly fatty acids are dissolved, and, what is much more important, rendered partially dialysable, by these constituents.

The most striking of the hydrotropic substances encountered in the body are the bile salts. The following results will illustrate the effect of the bile salts on the solubilisation of oleic acid in water. The oleic acid was emulsified in a phosphate solution of known *p*H, and then 5 per cent. sodium glycocholate added. The mixture was placed in a dialyser, outside which was the same mixture without any fatty acid. After sixteen hours' dialysis the contents of the dialyser and the outer fluid were analysed for fat, with the results shown:—

Diffusion of Oleic Acid dissolved in Na Glycocholate
(Verzár and Kúthy)

No.	*p*H	Per cent. oleic acid.				Per cent of fat not diffusible.
		In dialyser.		In outside fluid.		
		Beginning.	End.	Beginning.	End.	
1	6·5	0·081	0·052	0·0	0·042	—
2	6·5	0·250	0·172	0·0	0·078	37·6
3	7·0	0·059	0·033	0·0	0 027	—
4	7·0	0·220	0·142	0·0	0·069	37·3

The results show that with the more dilute solutions, the fatty acid was almost completely diffusible, but with the stronger ones, only about two-thirds of it was really dissolved, the remainder being in colloidal form, but this could all be got into a diffusible form if the outer fluid were changed repeatedly.

It cannot be doubted that the phenomena of hydrotropism play a very

* Neuberg. *Bioch. Ztschr.*, 1916, **76**, 105; Verzár. *Ergebn. d. Physiol.*, 1931, **32**, 421.

† Verzár and Kúthy, *Bioch. Ztschr.*, 1929, **205**, 369; **210**, 265, 281; 1931, **230**, 451; Kúthy, *Pflüger's Arch.*, 1930, **225**, 567; Verzár, *Ergebn. d. Physiol.*, **32**, 391.

important *rôle* in connection with the absorption of fats, sterols,* calcium salts, and other substances which are normally insoluble in water.

THE ABSORPTION OF FATS

The processes of digestion of fat result in the production of glycerol and fatty acids, if the reaction be neutral or slightly acid, as it commonly is. Analyses of the contents of the gut after a fatty meal show that the greater proportion of the fats are present as fatty acids, the amount of these as compared with unchanged fat increasing as we descend the gut. Much of the fat is thus presented for absorption in the state of a true solution of fatty acids in the bile salts, in consequence of the hydrotropic action of

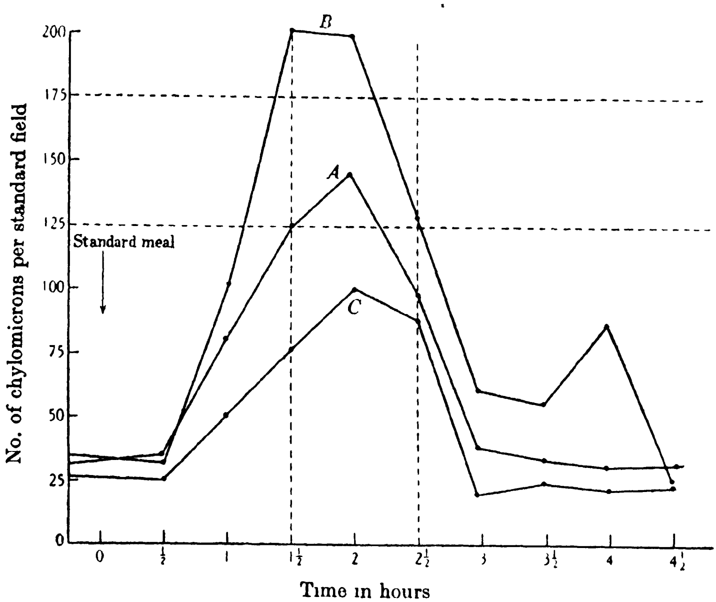

FIG 563. Counts of chylomicrons (chylomicrographs) of A, normal subject; B, fat subject; C, thin subject. Grid marked in dots indicates normal limits. (FRAZER and STEWART. *J Physiol.*, 1939, **95**, 23P)

the latter. Very little of it can be presented in the form of a soap, since soaps are unstable at reactions more acid than pH 9·0. Some seems to be absorbed without being split at all, but the means by which this is effected is unknown.

If an animal be examined a few hours after a meal rich in fats, the lymphatics of the intestine, the *cisterna lymphatica magna* and the thoracic duct, are seen to be distended with a milky fluid—*chyle*. The opacity is due to the presence of minute granules (0.05 to 1 μ) called *chylomicrons*. These consist, not of fatty acids, but of neutral fat.† The fat in such chyle may amount to over 6 per cent., so that in a moderate-sized dog 12 grammes of fat may be carried in the course of an hour from the intestine to the blood by this means.‡ Chylomicrons begin to increase in the blood about thirty minutes after a standard meal containing about 30 grammes fat, and reach a peak at about two hours, the peak being higher for fat subjects than for thin ones (Fig. 563). During fat absorption the plasma and serum are also

* GARDNER and GAINSBOROUGH. *Quart. J. Med.*, 1930, **23**, 465.

† ELKES, FRAZER and STEWART. *J. Physiol.*, 1939, **95**, 68.

‡ GAGE and FISH, *Amer. J. Anat.*, 1924, **34**, 1; FRAZER and STEWART. *J. Physiol.*, 1939, **95**, 21P, 23P.

milky. On standing, fat globules may rise like cream to the surface of the serum. Fat is found in a free state in this finely divided condition in the blood plasma so long as it is being absorbed from the intestine.*

The Blood Lipides. The blood lipides consist of fats, phospholipides and cholesterol. In the post-absorptive state the plasma contains about 600 mg. lipides per 100 c.c. Of this about 100 mg. is usually neutral fat, but this may be absent; phospholipides (calculated as lecithin) amount to about 250 mg./100 c.c.; cholesterol about 150 mg./100 c.c., of which half is free and half is in the form of esters. The red cells contain about 700 mg. lipides per 100 c.c., distributed roughly as follows: neutral fat, 0–150 mg.; phospholipides, 450 mg.; cholesterol (nearly all free), 175 mg.

Complete starvation causes an increase in all the blood lipides, but the rise is especially great in neutral fats if the subject is obese. After a meal containing fat the blood fat and phosphatides begin to rise after about two hours, and remain high as long as absorption of fat continues, say for a further two to seven hours. The cholesterol esters of the plasma also increase in fat absorption. Apparently during absorption more of the lecithin is present in

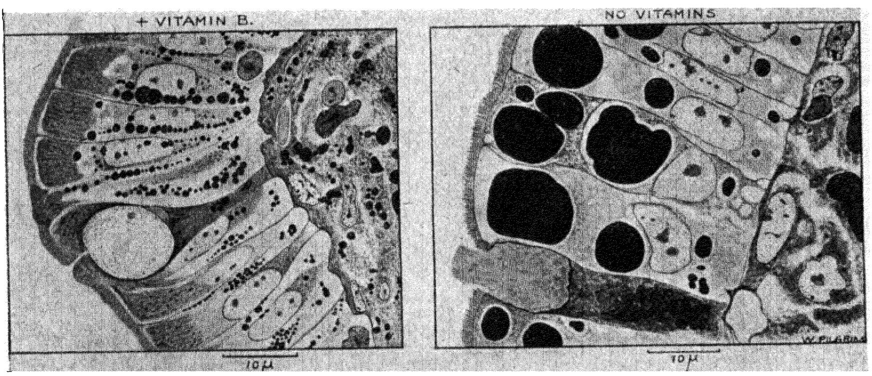

FIG. 564. Epithelium of villus during fat absorption in the rat. On left, showing absorption by streams. On right, absorption by drops. The fat is stained black with osmic acid. (MOTTRAM, CRAMER and DREW, *Brit. J. Exp. Path.*)

the corpuscles than in the plasma. In from six to ten hours after absorption is complete, free fat disappears, the serum becoming perfectly clear. Thus part at any rate of the fat which is absorbed from the gut is carried thence by the lymphatic channels in the form of neutral fat to the blood stream, by which it is distributed to the various tissues of the body, gradually leaving the blood stream in a manner which at present has not been determined. After a heavy meal of fat, and while the fat content of the blood is high, there appears to be engulfment of fat particles by the endothelial cells of the blood capillaries; the further fate of this fat is uncertain, but it may be passed on into the tissues.

Mode of Fat Absorption. Probably all the fat which is absorbed takes the path by way of the lymphatics and thus into the blood, though certain facts have presented difficulty in the way of accepting this simple view of the process. Ligature of the thoracic duct, if effective, certainly impedes the absorption of fat, but does not abolish it; the percentage of fat in the blood rapidly falls to a minimum which remains constant, even during starvation. If now fat be administered, although a considerable proportion of it may be absorbed, the percentage of fat in the blood is not raised. If

* NISSEN. "Studies in Alimentary Lipæmia in Man." (1933, Copenhagen: Levin and Munksgaard.)

the thoracic duct lymph be collected during the absorption of a given quantity of fat from the intestine, sometimes not more than 60 per cent. of the fat which has disappeared from the gut can be recovered from the lymph. Yet there is no evidence of an increase of fat in the blood of the mesenteric veins. These facts are perhaps explicable by the discovery by Lee * that the thoracic duct and receptaculum chyli have anastomotic connections which open into the azygos or other veins, by which means, when the thoracic duct is tied near its upper end, or when a cannula is inserted into it, a slow stream of lymph may nevertheless enter the blood circulation.

Microscopic examination of a section of a villus during fat absorption shows that the absorption occurs through the epithelial cells and in two different ways.† In the first method, called 'absorption by streams,' a stream of fine fat droplets passes on either side of the nucleus, and the outer part of the cell is free from droplets.‡ Abundant droplets of fat are also seen in the reticular tissue of the core of the villus (Fig. 564). It has been claimed that the cells of the intestinal mucosa, either surviving or fixed in formalin, when immersed in a solution of fatty acids in bile salts, become filled with fine granules of fatty acids.§ These subsequently are believed to be converted into neutral fat.

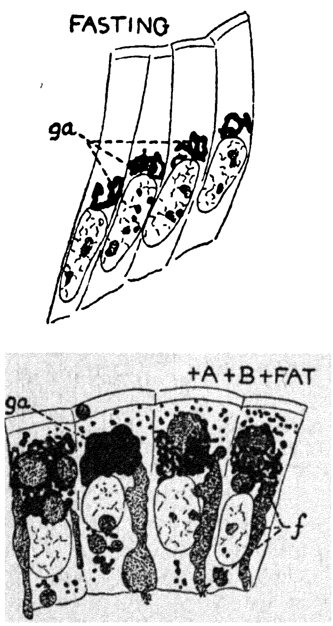

FIG. 565. Showing relation of Golgi apparatus to fat absorption. Above, fasting cells of villus. Below, cells during absorption of fat. *ga* = Golgi apparatus. *f* = fat droplet. (CRAMER and LUDFORD, *J. Physiol.*)

In the other method, called 'absorption by drops,' the columnar cells are found closely packed with fat granules (Fig. 564) which, small at the beginning of the process of absorption, rapidly enlarge till they occupy the greater part of the cell lying between the nucleus and the striated border. No fat globules are to be seen within the border itself. Mottram, Cramer and Drew found that when vitamins A and B were absent from the food, absorption was by drops. Frazer and Stewart's results suggest that absorption by drops occurs when unhydrolysed fat is absorbed direct.||

In the process of fat absorption, the fat is collected in the first place near the Golgi apparatus of the cell, which becomes greatly enlarged during the absorption, and no doubt plays some part in it (Fig. 565) ; Cramer and Ludford ¶ suggest that it effects the synthesis of neutral fat from fatty acid.

Investigations by special staining methods which distinguish fatty acids from neutral fat or soap have been made by Weiner.** He found that no soap was histologically detectable, but there was plenty of fatty acid, as well as neutral fat, in the epithelial cells, and fatty acid was still found even in the lacteals.

How the droplets of fat are passed from the interior of the epithelial cells into the reticular tissue of the villus, and from there onwards into the lumen of the lacteal, remains a mystery.

* LEE. *Johns Hopkins Hosp. Bull.*, 1922, **33**, 21.
† MOTTRAM, CRAMER and DREW. *Brit. J. Exp. Path.*, 1922, **3**, 179.
‡ MOTTRAM, CRAMER and DREW. *Brit. J. Exp. Path.*, 1922, **3**, 179.
§ ROSSI. *Arch. di. fisiol*, 1936, **36**, 365.
|| FRAZER and STEWART. *Physiol. Soc. Proc*, Feb., 1941.
¶ CRAMER and LUDFORD. *Journ. Physiol.*, 1925, **60**, 342.
** WEINER. *Ztschr. f. mikr. anat. Forsch.*, 1928, **13**, 197.

There is definite evidence* that the fat is not transported to any extent, as was once supposed, by leucocytes. It is conceivable that, if the epithelial cells, when sufficiently charged, extruded their droplets into the reticular tissue, these might be transferred thence into the lacteal, together with lymph, by the pumping action of the villi, since we have no positive evidence that the walls of the lacteals are continuous under all conditions.

In any case, the contractions of the villi must play an important part in the process by emptying the lacteals into the submucous plexus of lymphatics, so that the lymph in the spaces is constantly renewed and passes, laden with absorbed fat particles, into the valved lymphatics of the mesentery.

For the rapid absorption of fats, three factors are necessary, firstly the fat must be hydrolysed, secondly bile salts must be present, thirdly the enzyme phosphatase must be present in the gut: if any condition is lacking, absorption is delayed. The necessity for these conditions is evident, for although the bile does not dissolve neutral fats, it has a strong solvent action on fatty acids, on soaps, and even on the insoluble calcium soaps. This solvent power is greatest in the case of oleic acid, of which bile can dissolve 19 per cent. It is smaller in the case of pure stearic acid, but the solubility of the latter acid is largely increased if it be associated, as is usual, with oleic acid. It has been credibly suggested that the bile salts are preferentially absorbed, or adsorbed, by the epithelial cells covering the villi, and there exert their solvent action to great advantage.

The lecithin of the bile greatly augments the hydrotropic action of the bile salts.† Whereas a 5 per cent. sodium glycocholate solution dissolves 0·12 per cent. of oleic acid, the presence of 0·8 per cent. lecithin raises the solubility to 4·5 per cent.‡

We have seen that the bile salts poured into the gut undergo a circulation, being re-absorbed from the lower parts of the digestive tube, carried to the liver by the portal vein, and re-secreted in the bile. The same quantity of bile salts may therefore be used over and over again.

That the presence of bile in the intestine is essential for the normal absorption of fat is shown by the fact that on establishment of a biliary fistula, the utilisation of fat sinks from about 98 per cent. to about 40 per cent., the unabsorbed fat appearing in the fæces. This large undigested residue of fat also hinders the absorption of the other foodstuffs by covering them with an insoluble layer, so that nutrition as a whole may suffer considerably.

An important mechanism for fat absorption is the conversion of the fatty acids to phospholipide compounds by phosphorylation under the action of the intestinal phosphatase. This explains the increase in the phospholipides of the blood during fat absorption; some of the lecithin added to the blood is adsorbed by the red cells. When fat is given together with radioactive 'labelled' phosphorus compounds, the phospholipides of the intestinal mucosa in a short time contain the distinctive element. The importance of the enzyme phosphatase is shown by the fact that fat absorption is inhibited by the administration of phlorrhizin or iodoacetic acid, which poison this enzyme. Extirpation of the suprarenals also inhibits fat absorption,§ and appears also to upset the formation of phospholipides. Administration of either the cortical adrenal hormone, or of large amounts of NaCl, restore the

* LEACH. *J. Physiol.*, 1938, **93**, 1.
† MOORE and PARKER. *Proc. Roy. Soc.*, 1901, **68B**, 64.
‡ FÜRTH and SCHOLL. *Bioch. Ztschr.*, 1930, **222**, 430.
§ VERZÁR. *J. Physiol.*, 1935, **84**, 41P.

ability to absorb fat in adrenalectomised animals,* so that probably the lack of sodium salts is the fundamental factor which, after adrenalectomy, retards fat absorption.

Another channel for fat transport appears to be conversion into cholesterol esters instead of into glycerol esters.

Absorption may also be interfered with by ligature of the pancreatic duct and so removing lipase from the gut. If the fæces be analysed, it is found that a very large proportion of the fat has been split into fatty acids in the course of its passage through the alimentary canal. This lipolysis has, however, been carried out by the agency of micro-organisms, *i.e.* in the lower segments of the gut where the greater part of the bile has been already reabsorbed into the portal circulation. If fat, in a finely divided form such as cream or milk, be given to animals deprived of their pancreas, a certain proportion of it is absorbed. Under these conditions some lipolysis may occur in the stomach itself.

Substances which are physically almost identical with fats, *e.g.* liquid paraffin, are not necessarily absorbed, even when introduced into the intestine in the finest possible emulsion,† or else absorbed in small quantities only; after prolonged feeding with liquid paraffin small amounts of it can be found in the liver.‡ Cholesterol and other sterols, however, are absorbed, most readily when fat is also given, and may be recovered from the liver; the ease of absorption runs parallel with the solubility of the substance in bile salts.§ If neutral fat be melted with a soft paraffin and the resulting mixture made into a fine emulsion and administered, it is found that the intestine rejects the paraffin, but takes up the neutral fat. This result can be explained only by assuming that the fat in the particles has been actually dissolved out by the digestive juices and has been absorbed in a state of solution.

Under normal circumstances the utilisation of fat is almost complete. By the time the intestinal contents have arrived at the lower end of the ileum, 95 per cent. of the fat has been absorbed. Removal of the whole large intestine does not affect fat absorption.

THE ABSORPTION OF CARBOHYDRATES

As a result of the action of the various digestive juices, all the digestible carbohydrates of the normal diet of man are reduced to monosaccharides. In the absorption of these by far the greatest part is played by the small intestine. By the time that the food has arrived at the ileocæcal valve, practically the whole of the carbohydrate constituents of the food have been absorbed. All experimenters are agreed that the carbohydrates pass into the body by way of the vessels of the portal system. The lymph from the thoracic duct contains no more sugar than does the arterial blood taken at the same time, whereas there is an increased percentage of sugar in the portal blood, and even in the general circulation, during the absorption of a big carbohydrate meal.

The problem as to the passage of these substances across the intestinal wall into the blood vessels has already been dealt with. A most striking fact is the relative impermeability of the intestinal wall to the disaccharides as compared with the monosaccharides. The intestinal wall is apparently only able to take up to any extent such sugars as can be utilised by the cells

* Barnes *et al.* *Proc. Soc. Exp. Biol. Med.*, 1939, **40**, 651.
† Mellanby. *J. Physiol.*, 1927, **64**, Proc. v, xxxiii.
‡ Channon and Collinson. *Bioch. Journ.*, 1929, **23**, 676.
§ Channon and Collinson. *Bioch. Journ.*, 1928, **22**, 391.

of the organism. For this purpose the disaccharides are useless ; cane sugar or lactose introduced into the blood vessels or subcutaneously is excreted quantitatively in the urine. When maltose is injected in the same manner, a certain proportion of it is utilised owing to the fact that the blood and fluids of the body contain maltase, capable of converting the disaccharide into glucose. The absorption of these disaccharides occurs, therefore, much more slowly from the intestine than does the absorption of monosaccharides, the process of absorption being always preceded by the process of hydrolysis. Thus, huge doses of cane sugar may be taken without causing the appearance of cane sugar in the blood or urine. It has been found that glucose does not appear in the urine until as much as 320 grammes of cane sugar have been ingested, whereas any quantity of glucose over 100 grammes may give rise to slight glycosuria. Lactose is absorbed still more slowly.

THE ABSORPTION OF PROTEINS

The amino-acids produced by protein digestion are absorbed by the blood vessels of the intestinal villi, and pass unchanged into the portal blood. There is no significant increase in the amount of lymph flow or in the amount of protein contained in this lymph during digestion. Moreover, it was shown by Schmidt Mülheim that the absorption of proteins was not interfered with as the result of ligature of the thoracic duct.

During the absorption of a protein meal, changes have been described by various observers in the structures of the villus. Several observers have described changes in the epithelium as the result of protein digestion, the cells of the villi becoming swollen and vacuolated.

The first question we have to decide is whether the proteins are always completely hydrolysed before absorption. There is some evidence that serum proteins may be absorbed by the small intestine, though, unless unchanged protein can be shown to have passed into the blood, we cannot exclude the possibility that hydrolysis had occurred owing to the action of intestinal enzymes. Foreign proteins, such as egg albumin, or the serum of other species introduced into the blood stream, may cause poisonous effects and albuminuria. If injected in small quantities they excite, as a reaction on the part of the organism, the production in the blood serum of a *precipitin*, and later the animal becomes anaphylactically sensitised (Chapter IV.), and the presence of the precipitin or of anaphylactic sensibility may therefore be looked upon as a test by which we may decide whether these proteins have passed through the intestinal wall unchanged. In most cases it is found that, however abundant the amount of protein administered, none of it appears in the urine, nor is any precipitin formation or anaphylactic reaction aroused. Ascoli, however, has observed such results occasionally to follow the administration of large doses of egg white, and it has been shown * in the sensitised human subject that 'allergic' reactions may then ensue when the specific native protein is eaten, or even given per rectum. In experiments on guinea-pigs this was not the case unless there was simultaneous damage to the intestinal wall, or considerable pressure applied within the gut.† There is a difference, according to age, in the behaviour of animals to the introduction of soluble protein into their alimentary canal It seems that during the first few days of life the cellular lining of the alimentary canal is slightly permeable to foreign proteins, whereas later on little or no protein is absorbed unchanged from the gut.

The absorption of unchanged proteins can therefore play but a small part in the assimilation of protein as a whole.

When peptones and proteoses are introduced into the intestine they are rapidly broken down to amino-acids by erepsin of the intestinal juice ; this explains why, in the earlier experiments when peptone was absorbed no peptone could be found in the

* Süssmann, Davidson and Walzer. *Arch. Int. Med.*, 1928, **42**, 409 ; Brunner and Walzer *Arch. Int. Med.*, 1928, **42**, 172.

† Hettwer and Kriz. *Amer. J. Physiol.*, 1925, **73**, 539.

portal blood—a fact which led to the incorrect deduction that the peptone had, in the course of absorption, been synthesised to form native proteins of the blood plasma.

We know from the researches of Fischer and others that, although the different proteins in our food present a marvellous qualitative similitude, in that all of them yield on hydrolysis the same kinds of amino-acids, there are great differences in the relative amounts and stereo-chemical arrangement of these amino-acids in different proteins. It is difficult to imagine how, for instance, muscle protein could be formed from wheat protein, a process continually occurring in the growing animal, unless we assumed that the protein molecule is first partly taken to pieces, and that its constituent molecules are then selected by the growing cells of the body and built up in the order and proportions which are characteristic of muscle protein.

Very little energy is lost in such transformation, *i.e.* the heat of combustion of the products of digestion would differ from that of the original protein by less than 5 per cent. The energy of the protein as evolved in the body lies, not in the coupling of the amino-acids with one another, but is derived from the combustion of the carbon of the molecule into carbon dioxide.

Since all the evidence shows that ingested protein undergoes complete hydrolysis before entering the blood stream, it should thus be possible to feed an animal on a diet in which the protein had been replaced by the corresponding mixture of amino acids.

The experimental decision of this question was first attempted by O. Loewi,* who found that it was possible to keep a dog in a state of nitrogenous equilibrium on a diet containing fat, starch, and a pancreatic digest of protein which contained no substances giving the biuret test. These results have been confirmed for carnivora by Henderson and Dean,† by Abderhalden and London,‡ and by Henriques and Hansen.§ It has also been found that the intravenous injection of the digestion products will suffice.‖ The same result cannot be attained by the administration of the products of acid hydrolysis of protein,¶ because certain essential amino-acids are thereby destroyed.

We can conclude that the normal course of events is for the whole of the proteins of the food to be broken down to amino-acids prior to its entry into the blood.

The Fate of the Amino-Acids after Absorption. During a condition of starvation the normal protein requirements of the active tissues are met at the expense of the less active tissues. The proteins characteristic of any tissue can be taken down, removed to another part of the body, and built up into the proteins characteristic of some other active tissue. It is difficult to conceive that such a transference and transformation could occur in any other way than by a more or less thorough disintegration of the protein molecule at one place and its synthesis at the other, and we know that every tissue contains intracellular enzymes which are capable of effecting the disintegration of the protein molecule, and are responsible for the autolytic degeneration of tissues after death. If, therefore, the normal interchange of protein between the tissues is accomplished by the disintegration of the proteins into their constituent amino-acids and their subsequent reintegration, there is no *a priori* reason to believe that the blood carries the proteins from the alimentary canal to the tissues in any other form than that of amino-acids. Strongly in support of this conclusion is the evidence ** that

* LOEWI. *Arch. f. exp. Path.*, 1902, **48**, 303.

† HENDERSON and DEAN. *Amer. J. Physiol.*, 1903, **9**, 386.

‡ ABDERHALDEN and LONDON. *Z. f. Physiol. Chem.*, 1907, **54**. 80.

§ HENRIQUES and HANSEN. *Z. f. Physiol. Chem.*, 1906, **49**, 113.

‖ HENRIQUES and ANDERSEN. *Z. f. Physiol. Chem.*, 1913, **88**, 357.

¶ HENRIQUES. *Z. f. Physiol. Chem.*, 1907, **54**, 406.

** CATHCART and LEATHES. *J. Physiol.*, 1905, **33**, 462 ; FOLIN and DENIS, *J. Biol. Chem.*, 912, **11**, 87 ; **12**, 141.

after a protein meal there is an increase in the non-protein nitrogen (*i.e.* the nitrogen not present as coagulable protein) of the blood, and particularly in the portal vein blood. The final proof was rendered possible by Van Slyke's method * for the detection of small quantities of amino-acids in the blood and tissues, and ultimately by the actual isolation of amino-acids from the dialysate prepared by circulating blood from a living animal through a collodion dialyser,† and back to the animal again. Several different amino-acids have been isolated from the blood,‡ though it is not known to what extent or under what circumstances the individual amino-acids are altered in amount. In sum, however, their amount does vary. Van Slyke and Meyer § showed that the blood always contains a certain amount of amino-acids, even during fasting. After a protein meal there is a considerable increase in the amount of amino-acids. Thus the blood of fasting animals contains from 3 to 6 milligrammes of amino-acid nitrogen per 100 c.c. Blood taken after food contains from 8 to 10 milligrammes of amino-acid nitrogen per 100 c.c.

The question of the fate of amino-acids thus absorbed from the intestine into the blood is decided by an estimation of the amino-acid content of the blood and of different tissues at various times after the injection of amino-acids into the blood. Van Slyke and Meyer found that after the injection of amino-acid, only a certain proportion is excreted with the urine, and that the rest of the amino-acid rapidly disappears from the blood and is taken up by the tissues without undergoing any immediate chemical change, though in the case of certain tissues, such as the muscles, a definite saturation point exists which sets the limit to the amount of amino-acids that can be absorbed. On the other hand the capacity of the internal organs, and especially of the liver, for the absorption of amino-acids is much greater.

It is worthy of note, however, that the absorption of amino-acids by the tissues from the blood is never complete, *i.e.* the amino-acids of the blood must be in a state of equilibrium with those of the tissues, although the concentration in the latter may be much greater than in the former. If several hours be allowed to elapse after the injection of amino-acids before the analysis of the tissue is undertaken, it is found that the amino-acid nitrogen content of the liver may have returned to normal, although the concentration in the muscles has suffered no appreciable fall. Since we have evidence that the circulation of amino-acids through the liver gives rise in this organ to the formation of urea, we must conclude that this organ is especially responsible for the breakdown of the amino-acids which are not directly required for replacing tissue waste. This breakdown must involve a process of deamination.

We therefore conclude that the amino-acids produced by protein digestion are absorbed without further change into the blood stream. They then circulate throughout the body, as a result of which an equilibrium concentration is established for each amino-acid between the blood and the several tissues, a certain proportion of them finally being built up in each tissue into the proteins characteristic of that tissue in order to replace the waste caused by wear and tear. The rest, probably the major part of the amino acid, is taken up by the liver, where most of it undergoes deamina-

* Van Slyke. *J. Biol. Chem.*, 1911, **9**, 185 (original form); *ibid.*, 1929, **83**, 425 (improved form). A colorimetric method has been given by Folin, *J. Biol. Chem.*, 1922, **51**, 377.

† Abel, Rountree and Turner. *J. Pharmacol.*, 1914, **5**, 275.

‡ Abderhalden. *Z. f. Phys. Chem.*, 1913, **88**, 478.

§ Van Slyke and Meyer. *J. Biol. Chem.*, 1913, **16**, 197, 213, 231.

tion, the nitrogen moiety being rapidly converted into urea which is excreted by the kidneys, while the non-nitrogenous moiety is carried to the working tissues, which it serves as a ready and immediate source of energy.

The distribution of the amino-acids in fasting, starving and during absorption has been studied in cats by Bolton and Payling Wright.* They estimated the amino-nitrogen in blood and in plasma coming from the intestine by the superior mesenteric vein, in that leaving the liver by the hepatic vein, in blood going to the tissues by the carotid artery, in blood leaving the tissues by the inferior vena cava, and in the lymph.

Some of their average results are given below :—

Condition	Average amino-N(mg/100 cc) in blood plasma of				Lymph
	Carotid	Sup Mes Vein	Hepatic vein	Inf ven cava	
Fasting . . .	5·0	5·3	4·8	5·3	5·9
Starving . . .	4·9	4·9	4·4	5·4	6·1
Fed peptone 20 g. 5 hrs. before . . .	15·2	19·5	15·9	14·9	17·1

These experiments bear out the current view that absorption of the amino-acids into both the capillaries and the lymphatics of the villi is in accordance with the laws of diffusion. The blood capillaries being more superficial, and the lymph being diluted by the tissue fluid derived from the afferent blood stream, the amino-acids reach a higher level in the blood than in the lymph during absorption. Always there is a lower level of amino-acids in the hepatic vein blood, showing that the liver is constantly removing amino-acids from the blood. Also, in fasting, the blood from the tissues and intestine has the same content of amino-acid, while in starvation the venous return from the tissues has a raised content as compared with the arterial blood, which shows that the muscles and other tissues are undergoing a process of breakdown, yielding amino-acids to the blood and lymph.

In Fig. 566 are shown the results of experiments on rats absorbing a diet of meat. The upper curve (I) shows the per cent. of absorption from the intestine ; the lower curves show (II) the amino-nitrogen of the blood, and (III) the oxygen consumption of the animal. During the first three hours absorption is rapid, the amino-N of the blood rises steeply, and with it the oxygen usage—a similar increase of oxygen usage is obtained if amino-acids are injected direct into the blood (specific dynamic action).

The fact that not only the blood but also the tissues contain amino-acids, even after complete starvation for some days, shows that these substances are intermediate steps not only in the synthesis but in the breaking down of body proteins. Free amino-acids are thus the protein currency of the body, just as glucose is the carbohydrate currency. In the fasting body we must regard the processes of autolysis as the main source of the amino-acids found in the tissues, and it is by autolysis that the proteins of the resting tissues are made available in

* Bolton and Payling Wright. *J. Physiol.*, 1937 **89**, 269.

starvation for those whose continued working is essential for the maintenance of life. The fact that high protein feeding does not appreciably increase the amino-acid content of the tissues shows that any storage of

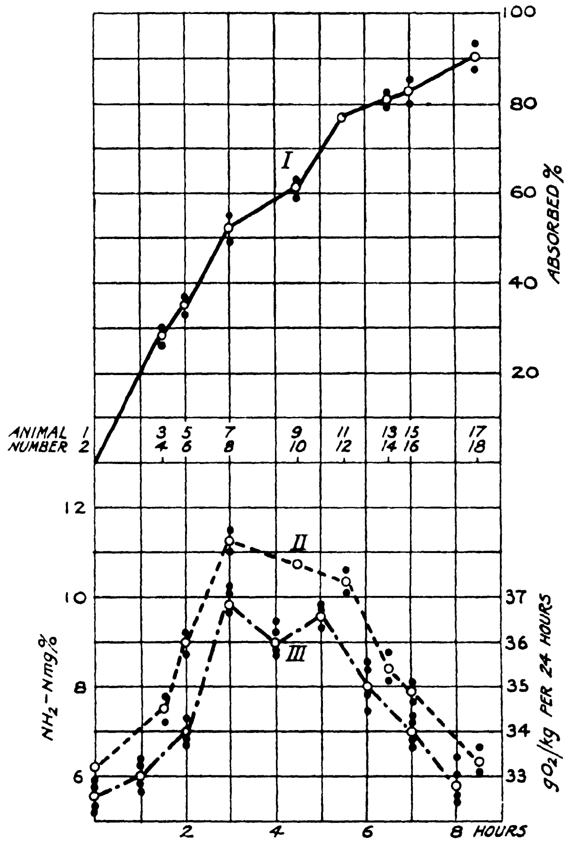

FIG 566. Effect of feeding rats with meat. I Rate of absorption from alimentary canal. II. Amino-N of blood. III. Oxygen usage of animals. (KÚTHY.) (From VERZÁR, *Ergebn. d. Physiol.*, **32**, 1931.)

nitrogen in the organism must take place, not in the form of amino-acids, but as body protein.

The Plasma Proteins. The plasma proteins, serum albumin and serum globulin, probably play an important *rôle* in protein metabolism. The plasma proteins are almost certainly formed, in the liver, from the amino-acids derived from digestion of protein ; they then act as a reserve of readily available protein in case of need.

The plasma proteins can be experimentally lowered by the process called *plasmapheresis.* In this, blood is drawn daily, and the corpuscles washed with isotonic salt solution and reinjected. By suitable, steadily diminishing, daily plasmaphereses the serum proteins can be lowered, say, from 6 per cent. to a certain low level, say, 4 per cent. The plasma protein that must be removed per week in order to maintain this level reaches a constant figure, called the basal output. If anything is done to upset the balance, more or less plasma protein must be removed in order to keep the constant low level. Feeding with different proteins, for instance, will alter it. Thus, 100 grammes

ox serum protein will form 38 grammes serum protein in a dog, 100 grammes meat forms about 20 grammes, and 100 grammes gelatin only 5 grammes.

Plasma protein can be formed also from reserve stores in the body, and fasting animals can replace 40 to 60 per cent. of the circulating plasma protein within twenty-four hours. Conversely, plasma protein can be utilised for general purposes. Thus a protein-starved dog can be kept in nitrogenous equilibrium by frequent injections of dog plasma. This accession of protein appears to benefit the tissues directly, for it is not followed by an increase of nitrogen output as dietary protein would be, nor does it raise the blood amino-acids, although the injected plasma protein has all disappeared in less than twenty-four hours.

It seems probable, therefore, that the tissue proteins are supported by amino-acids and/or plasma proteins. Part of the tissue protein forms a reserve and is readily and quickly depleted. Supplies of amino-acid from without, and demand for protein within, are in a condition of dynamic equilibrium with protein reserves. If the need for protein for cells or plasma exceeds the supply from amino-acids, the reserve stores including plasma proteins will be depleted, and if less it will be replenished. It seems probable that tissue proteins can be built from plasma proteins without loss of nitrogen.

THE ACTUAL COURSE OF DIGESTION

London * described the course of digestion of meals of various characters in dogs which had been provided with fistulæ in one of the following places: (*a*) gastric fistula (into the body of the stomach); (*b*) pyloric fistula (on the duodenal side of the pylorus); (*c*) duodenal fistula (about 1 foot below the pylorus); (*d*) jejunal fistula (about the middle of the small intestine); (*e*) ileal fistula (just above the cæcum).

We may take as an example the course of digestion of a meal composed of 200 grammes of bread. This is eaten by the animal, mixed with the saliva and swallowed. On arriving in the stomach it gives rise to the secretion of gastric juice. On the average 200 grammes of bread evoked the secretion of 20 grammes of saliva, about 10 grammes of mucus from the coats of the stomach, and about 315 grammes of gastric juice. The secretion of gastric juice is continuous during the whole time that the food remains in the stomach. In the animal with a pyloric fistula, one to two minutes after the meal had been taken, a few drops of alkaline fluid were extruded from the opening. From three to eight minutes after the conclusion of the meal, small quantities of clear acid gastric juice were repeatedly extruded. The first admixture of the food with the outflow from the fistula occurred at eight to twelve minutes after the completion of the meal, and after this time the pylorus continued to open at regular intervals of ten to forty seconds, discharging each time a small amount of fluid composed of particles of undigested bread mixed with gastric juice. One and a half hours later the pylorus began to open less regularly and the fluid became of a more pasty consistence, devoid of lumps of undigested bread. In the fourth, fifth and sixth hours after the meal, the pylorus opened only once every one or two minutes, and towards the end of this period the fluid extruded was clear. The following Table shows the percentage amount of food taken which had left the stomach at the end of each hour after the meal:

First hour	32·6	per cent.
Second hour	17·9	,,
Third hour	29·5	,,
Fourth hour	1·87	,,
Fifth hour	6·66	,,
Sixth hour	4·21	,,

The large proportion of the ingested food leaving the stomach during the first two or three hours can hardly be regarded as normal. Since in these experiments there was

* London. *Z. f. Physiol. Chem.*, 1907, **53**, 246; 1908, **56**, 378; 1909, **60**, 191, 267.

a free outflow from the pylorus and the food was not allowed to enter the duodenum, the normal slow emptying of the stomach was interfered with. The gastric contents obtained in this way were analysed in order to find what changes had been wrought on the food by the gastric juice. It was found that 32 per cent. of the bread had been brought into solution. This solution had affected the proteins more than the carbohydrates. Thus 67 per cent. of the nitrogen had been brought into soluble form, consisting chiefly of proteoses and peptones. No amino-acids were formed. Only 25 per cent. of the starch of the bread had been rendered soluble, and of this, 21 per cent. was in the form of dextrin and 4 per cent. in the form of sugar. No absorption, however, either of the digested proteins or of the digested carbohydrates was ever found to take place in the stomach.

The influence exerted by the pancreatic juice, bile and succus entericus poured out on the food in the duodenum was studied by analysis of the intestinal contents leaving the intestine by a fistula, either at the lower end of the duodenum or in the jejunum or in the ileum. From the duodenal fistula the expulsion of food occurs at repeated intervals but in a somewhat irregular fashion, its movements being determined partly by the contractions of the stomach and partly by those of the duodenal wall. Usually a large gush is followed by a series of small gushes. Although only a foot intervenes between the duodenal fistula and the pyloric fistula, a great difference is observed in the character of the intestinal contents obtained in the two cases. The outflow from the duodenum, being mixed with the pancreatic juice and the bile, is yellow in colour and increased in amount. With a meal of 200 grammes there are secreted on the average 130 grammes of bile and 140 grammes of pancreatic juice. During its passage through the duodenum the carbohydrates of the food undergo considerable changes, so that even one foot below the pylorus we find that one-half to three-fifths of the carbohydrates have been converted into dextrin and sugar. A further digestion of the proteins also takes place amounting to about one-tenth of the whole protein taken with the food.

On deducting the amount of juices which have been added to the food, it is found that even in this short length of intestine absorption has taken place of about one-sixth of the ingested food, about one-fourth of the carbohydrates having been absorbed and about one-eighth of the proteins.

In a dog with a fistula about the middle of its small intestine, the outflow began six to fifteen minutes after the meal, and lasted six or seven hours. The outflow was by small gushes repeated at intervals of five to ten seconds, separated by intervals of one to five minutes during which nothing appeared at the orifice of the cannula. The material obtained was quite different in character from that flowing from the duodenal fistula. The pasty character had disappeared, the material forming a frothy, orange-yellow, even jelly-like mass with practically no trace of undigested bread.

From a fistula in the ileum the outflow occurred at long intervals of three to fifteen minutes and was much scantier than that obtained from the jejunal fistula, consisting of a thick jelly-like, orange-coloured mass. Both proteins and carbohydrates were entirely digested, and in the case of the former the chief products of digestion consisted of amino-acids. Thus in one experiment, after four large meals of 500 grammes of meat each had been given in order to obtain sufficient quantity for analysis, 175 grammes of soluble substances were obtained. From this were isolated tyrosine, leucine, alanine, aspartic acid, lysine, and traces of arginine and histidine.

From a fistula in the cæcum there was no outflow until four or five hours after the meal had been taken. The material from the gut was then extruded in fæcal-like masses at long intervals of one half to one hour. This regular outflow lasted for about six hours. The reaction of the contents was strongly alkaline, with no food particles, and the material contained merely débris of cells, with small traces of sugar, dextrin and unaltered starch. The absorption of the foodstuffs is thus practically complete by the time that the food has reached the lower end of the small intestine.

The following Table gives the total amounts obtained in a series of experiments from the different fistulæ after administration of 200 grammes of bread, and also the percentage amount of foodstuffs which had been absorbed before the food had arrived at the level of the fistula in question :

	Total amounts obtained from 200 grms. of bread.	Absorbed, per cent.
Pyloric fistula . .	691 grammes	0
Duodenal fistula . .	691 ,,	17·45
Jejunal fistula . .	585 ,,	37·77
Ileal fistula . . .	412 ,,	67·65
Cæcal fistula . . .	80 ,,	94·34

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CHAPTER XLVI

THE HISTORY OF THE FOODSTUFFS

THE USE OF ISOTOPES IN THE STUDY OF METABOLISM

An important aid in following the fate of a substance in the body is provided by the use of elements in isotopic form. There are two types of isotopes which may be used. The first of these are the naturally occurring ones.

When an element occurs in both normal and isotopic forms, these differ only in atomic weight, and physical properties which depend on it, but are otherwise indistinguishable. The proportion of an isotope in a given element is constant, whether the element be free or in combination. Thus nitrogen, whether in air, in an amino-acid, ammonia, urea or protein, always contains 1 part of N^{15} (*i.e.* nitrogen of atomic weight 15) to 269 parts of the normal nitrogen N^{14}. Similarly, heavy hydrogen, or deuterium (D), of atomic weight 2, occurs with normal hydrogen in the proportion of 1/6000, and heavy oxygen, O^{18} with O^{16} in the ratio 1/530. The reason for this constant composition is that none of the agencies of nature, biological or otherwise, can discriminate between, and so in any way fractionate, the mixtures of elements and isotopes. Depending on differences in physical properties, however, an artificial separation can be effected, and so the element or its compounds can be prepared which will contain the isotope in a higher proportion than that found naturally. The amount of an isotope in such an element or compound is capable of determination with accuracy by physical methods, such as determination of density, mass spectrographic analysis, etc. Thus the concentration of N^{15} in nitrogen can be determined to within 0·003 per cent.

The other type of isotope is the artificially prepared radioactive form of the element; thus carbon, phosphorus, calcium, sulphur, etc., can be rendered radioactive, and, since the active element can readily be identified and estimated by this property, the element, whether free or in a compound, can be traced through the body. These forms of elements are also biologically indistinguishable from the normal forms.

As an example of the use of natural isotopes we may mention the employment of N^{15} in the study of the fate of the amino-group of amino-acids. When amino acids, the nitrogen of which has been labelled by the addition of the N^{15} compound, are given with the diet, most of the nitrogen was recoverable in combination in the tissue proteins, and not in the excreta.

As an instance of the use of a radio-active element, we may quote experiments with labelled phosphorus, prepared from sulphur by neutron bombardment. When this was injected subcutaneously into rabbits, as sodium phosphate, it was found that within twenty-seven days 45 per cent. of it was excreted into the urine, and 11 per cent. in the fæces, and the element was shown to be present even in the calcium phosphate of the bones for a few weeks.

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1. PROTEIN METABOLISM

The proteins taken in with the food have a twofold destiny. One part, usually the greater part, is broken down and yields energy, as do also the fats and carbohydrates.

Part of them, and normally the smaller part, however, ultimately forms an indispensable constituent of the living substance. This part cannot be replaced by other foodstuffs, and we will consider its history first.

A. PROTEIN SYNTHESIS

The proteins which form an essential part of the living protoplasm must be derived from the proteins of the food; but we can be quite certain that their formation is no question of mere translation of a substance from one place to another. For we have seen, in the first place, that the ingested proteins are broken down more or less completely into their constituent amino-acids before being even absorbed into the blood stream. Furthermore, the tissue proteins are in practically every case chemically different from those which are ingested, varying, as they do, not only from tissue to tissue, but from one species of animal to another.

The course of the synthesis of body proteins has not been followed: it has been shown that amino-acids are abstracted from the blood by the tissues. But the only certain fact in a sea of speculation is that the nitrogen of the protein so formed is derived from the amino-acids of the blood.

It has been supposed, not without reason, since the digestion of protein consists in the disintegration of the molecule down to the constituent amino-acids, that the resynthesis of protein need only be a reversal of that process, in which suitable amino-acids are re-combined. Indeed, the formation of plasteins by the action of pepsin on the hydrolysis products of proteins has been urged as a clear demonstration of the possibility of the enzymic synthesis of proteins. This evidence is, however, far from satisfactory, since the substance produced is a relatively simple one, and the starting-point not amino-acids.

The fact that certain of the amino-acids are essential for growth has been advanced in further support of the view that protein formation is largely a re-assembling of ready-made amino-acids; according to this, the non-essential amino-acids can, if necessary, be synthesised in the body, so that if the essential ones are supplied ready made, the jig-saw molecule may be duly built up.

There is another possibility, however. The body may be quite well able to synthesise all the amino-acids in amount sufficient to form its proteins, but may require the essential ones at times of growth for quite different purposes, and in larger amount than it can make. It has been stated, for instance, that when animals are fed on a diet lacking in tryptophan, the injection of that substance subcutaneously gives no relief from the symptoms; for this to occur, the tryptophan must be absorbed, and pass the liver. Lysine also, though necessary for growth, is not essential for maintaining life: yet presumably some of the lysine must need to be renewed in maintenance processes.

The Synthesis of Amino-Acids. If the body can effect deamination of an amino-acid, there seems no reason why it should not, by reversal of the enzyme action involved, synthesise an amino-acid from its corresponding ketonic acid and ammonia. Knoop * found that the next higher homologue of phenyl alanine, namely phenyl-α-amino-butyric acid, when administered to an animal, was excreted in large quantities in the

* KNOOP. *Z. f. Physiol. Chem.*, 1910, **67**, 489; 1914, **89**, 151.

urine as an acetyl derivative, which was easily isolated in a state of purity. Knoop found that the administration of phenyl-α-keto-butyric acid led to the excretion of the same acetyl derivative in the urine. Since keto-acids are ordinary products of the breakdown of amino-acids, it is evident that the animal body can add ammonia and form amino-acids from them.

If the phenyl derivative of the corresponding hydroxy-acid, α hydroxy-butyric acid, was administered, this was also excreted as the acetyl amino-compound. This and other evidence suggests that amino-, keto- and hydroxy-acids are interconvertible in the body, the connecting link being the keto-acid, *e.g.*

$$\underset{\text{Alanine}}{CH_3 . CHNH_2COOH} \rightleftarrows \underset{\text{Pyruvic Acid}}{CH_3CO . COOH} \rightleftarrows \underset{\text{Lactic Acid}}{CH_3CHOH . COOH}$$

These changes can be effected by the perfused liver as readily as by the whole body.*

The keto- or hydroxy-acids need not be derived from proteins at all but, like lactic acid, may result from carbohydrate metabolism. Thus, part of the nitrogen set free by protein disintegration might be recombined with the formation of amino-fatty acids, without giving rise to urea or appearing in any way in the nitrogen balance-sheet of the body. This possibility enjoins the necessity of caution in interpreting the results of metabolism experiments, where the nitrogen excreted is taken to represent the total protein metabolism of the body.

Are the Amino-acids interconvertible ? Glycine and alanine can be easily manufactured in the body, but although the animal organism is apparently capable of synthesising amino-acids from ammonia and the corresponding keto- or hydroxy acid, it is unable to convert one amino-acid into any other. On this account many proteins are inadequate as food substances since they do not contain the necessary amino-acid groups.

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B. PROTEIN CATABOLISM

The proteins of the tissues ultimately become broken down, as do those which never become built up into tissue proteins. This tissue metabolism accounts for a small part only of the nitrogen of the food. The more protein taken in with the food the greater is the relative amount which is applied to the energy needs of the body. To find out what are the end products of the tissue metabolism, we should therefore reduce the protein in the diet to its minimum. If we compare the composition of the urine obtained on a diet containing a normal quantity of protein and on a diet containing a minimal amount, we find evidence of a qualitative difference as is well brought out in the Table given here.† On high protein diet the greater

DISTRIBUTION OF NITROGEN IN URINE ON VARIOUS DIETS

	July 13 Ordinary diet	July 20 Low protein diet
Vol. of urine	1170 c.c.	385 c.c.
Total nitrogen	16·8 grm.	3·60 grm.
Urea	14·70 grm. = 87·5 %	2·20 grm. = 61·7 %
Ammonia	0·49 grm. = 3·0 %	0·42 grm. = 11·3 %
Uric acid	0·18 grm. = 1·1 %	0·09 grm. = 2·5 %
Creatinine	0·58 grm. = 3·6 %	0·60 grm. = 17·2 %
Undetermined	0·85 grm. = 4·9 %	0·27 grm. = 7·3 %
Total SO_3	3·64 grm.	0·76 grm.
Inorganic SO_3	3·27 grm. = 90·0 %	0·46 grm. = 60·5 %
Ethereal SO_3	0·19 grm. = 5·2 %	0·10 grm. = 13·2 %
Neutral S	0·18 grm. = 4·8 %	0·20 grm. = 26·3 %

* EMBDEN and SCHMITZ. *Biol. Ztschr.*, 1910, **29**, 423 ; 1912, **38**, 393.
† FOLIN. *Amer. J. Physiol.*, **1905**, **13**, 45, 66 117.

part of the nitrogen of the urine is derived directly from the food, whereas on restricted protein a relatively larger proportion of it must come from protein of the tissues. Folin distinguished these two sources of the nitrogen of the urine as *exogenous*, *i.e.* that from the food, and *endogenous*, *i.e.* derived from the tissues. Two facts stand out in comparing these two urinary analyses. In the first place, in a normal protein diet the urea accounts for 87 per cent. of the total nitrogen of the urine. On an excessive protein diet this percentage may rise to 90 or 95. On a low protein diet, not only is the total amount of nitrogen in the urine much less, but the percentage of nitrogen appearing as urea is reduced to 60. On the other hand, practically identical amounts of creatinine are obtained under the two conditions; on the full diet it amounts only to 3·6 per cent., on the low protein diet it forms as much as 17 per cent. of the total nitrogen output.

Urea Formation. We are therefore justified in regarding urea as to a

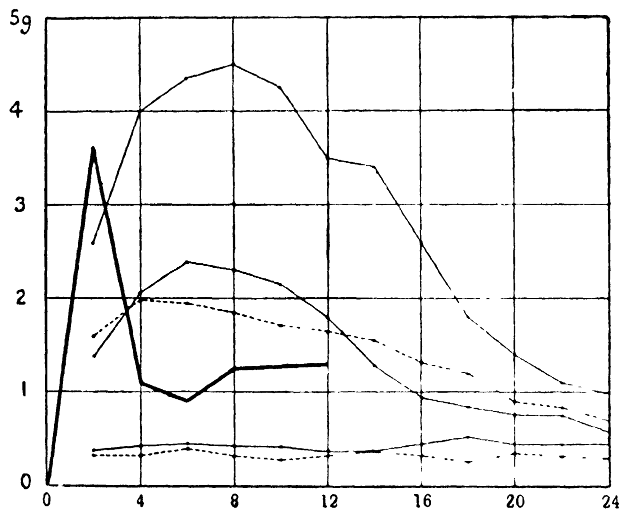


FIG. 567. The Hourly Variation in the Excretion of Nitrogen after a Meal. The meal was given at 0. The thick line represents the average absorption of the food from the alimentary canal. The thin-lined curves, in order from above, represent the N excretion (1) after a meal of 1000 grammes meat; (2) after 500 grammes meat and 150 grammes fat; (3) after a meal of 500 grammes meat; (4) and (5) both represent the excretion in a fasting animal. (From TIGERSTEDT after FEDER.)

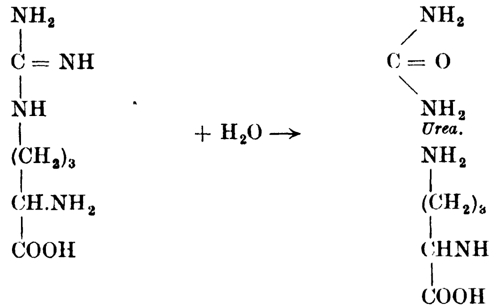
large extent exogenous in origin, and as derived directly from the nitrogen of protein molecules, which may not at any time have formed part of the living tissues of the body.

On giving a large protein meal to a dog, the urea in the urine rapidly rises, and at the end of four or five hours 50 per cent. of the total nitrogen ingested with the food has appeared in the urine as urea (Fig. 567). If we take into account that the digestion of a meat meal in this animal may go on for eight hours, we are justified in the statement that by far the greater portion of the protein nitrogen taken with the food is excreted as urea in the urine almost directly after absorption.* Urea is therefore to be regarded in the first place as an index to the amount of protein absorbed. That amino-acids are the immediate precursors of the urea is shown by the fact

* PETTENKOFER and VOIT. *Ann. Chemie.* 1862, 52, 361.

that the administration of these bodies is followed very rapidly by the appearance of the whole of their nitrogen in the urine as urea.*

The formation of urea from one of the amino-acids, viz. arginine, is accomplished in a very simple fashion. By the action of the enzyme *arginase*, which is abundant in the liver, arginine is split up into urea and ornithine † :



Arginine *Ornithine*

As will be seen shortly, this reaction has considerable significance. In other instances the conversion is less direct, and is now known to be even less simple than had been formerly supposed. The first stage is the formation of ammonia. If amino-acids be treated with the pulp of various organs, there is a production of ammonia. Leucine, glycine, tyrosine and cystine give rise to ammonia, while none is produced from phenylalanine. The production of ammonia is due to the presence of deaminising enzymes in the cells of various tissues. According to van Slyke, the liver plays the chief part in the breakdown of amino-acids, though there is reason to attribute the possession of slight powers to other tissues, *e.g.* the muscles. As a result of this deamination ammonia is set free and is rapidly converted into urea in the liver. That ammonia is the immediate precursor of urea is shown by the fact that no other nitrogenous substance, *e.g.* cyanic acid, yields urea in the liver more rapidly than does ammonia.

Although there is normally a small amount of ammonia in the urine, it is not increased by injection or administration of ammonium carbonate which gives rise simply to a corresponding increase in the urea of the urine.‡ A large body of evidence points to the liver as being the chief seat of conversion of ammonia into urea. Thus Schröder§ showed that if defibrinated blood mixed with ammonium carbonate was passed for one hour through a surviving liver, the ammonium carbonate disappeared and its place was taken by urea.

The ammonium carbonate may be regarded as losing 2 molecules of water, as a net result, as follows :

$$CO\begin{cases} ONH_4 \\ ONH_4 \end{cases} - 2H_2O = HN:C\begin{cases} NH_3 \\ | \\ O \end{cases}$$

though, as shown, below, the change is not so simple as this.

Confirmatory evidence of this removal of ammonia by the liver was supplied by Schroder's experiments on birds, but these are now only of historical interest, since the ammonia is converted in these animals not into urea, but into uric acid. The liver of birds can, for anatomical reasons, be readily extirpated, but in mammals the operation

* LEVENE and MEYER. *Amer. J. Physiol.*, 1909, **25**, 214.

† KOSSEL and DAKIN. *Z. f. physiol. Chem.*, 1904, **41**, 321.

‡ KNIERIEM. *Z. f. Biol.*, 1874, **10**, 263.

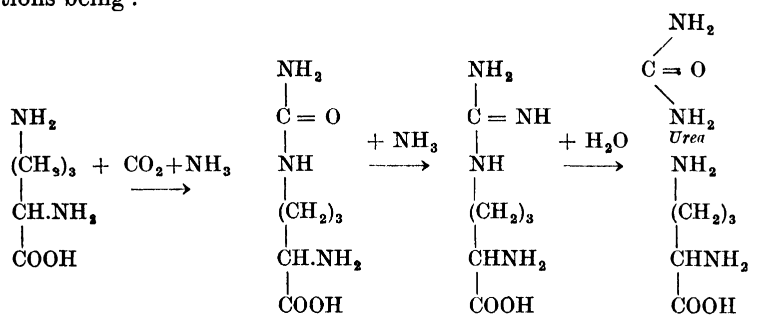
§ SCHRÖDER, *Arch. f. exp. Path.*, 1882, **15**, 364 ; LÖFFLER, *Bioch. Ztschr.*, 1918, **85**, 230.

is more difficult. The operation known as the Eck fistula, in which the portal vein and the inferior vena cava are joined together side by side, and the portal vein then tied at its entry into the liver, enables the portal blood to be diverted from the liver, but since the liver still has an arterial supply, this operation is not equivalent to removal of the liver, and, in the present connexion is also of merely historical interest. By indirect procedures, however, Mann * has succeeded in actually removing the liver from dogs.

After extirpation of the liver in dogs, both the deamination of the amino-acids and the formation of urea cease completely, indicating that under normal conditions both these processes are carried out in the liver. The animals only survive the completed operation for a few hours, but during that time the urea content of the blood and urine steadily falls. If the kidneys are also removed, the urea of the blood remains constant. In the normal animal, amino-acids are rapidly changed to urea, but when amino-acids are injected into the blood after hepatectomy, the greater part is recoverable from the blood and tissues, and there is no rise in urea or ammonia.

In man the evidence is necessarily indirect, and some believe that urea may be formed in other tissues besides the liver.

The actual steps by which ammonia is converted into urea are complex and, according to Krebs, depend upon the catalysing action of small amounts of ornithine, and on the presence of arginase.† In the liver, ornithine combines with ammonia and carbon dioxide to form first citrulline, and then with a molecule of ammonia to form arginine, the sum of the two reactions being :—



Ornithine *Citrulline* *Arginine* *Ornithine*

The arginine is then decomposed into urea and ornithine as explained above, and the ornithine thus set free can repeat the reaction.

Deamination. We thus see that the urea, which appears in the urine so rapidly after the ingestion of protein, does not signify a total disintegration of the protein molecule, but is merely the result of the throwing off of the nitrogenous part of the protein molecule by a process of deamination.

The investigation of the stages in deamination, and indeed in the disintegration of aliphatic derivatives generally, is rendered difficult by the fact that all the intermediate products undergo further change and leave the body in a state of complete oxidation as carbon dioxide and water. If, however, an amino-acid group be conjugated with an aromatic compound, *e.g.* forming a side chain of the benzene ring, it is protected from complete oxidation by the stability of this ring. The oxidation of the fatty side chain may proceed to a certain degree, so that intermediate products of metabolism may be excreted still attached to the benzene nucleus. In the α-amino-acids the point where disintegration first occurs is the α-group. Deamination is

* MANN, *Amer. J. Physiol.*, 1921, **55**, 285 ; MANN and MAGATH, *Ergebn. d. Physiol.*, 1924, **23**, 212 ; SOSKIN, *J. Lab. Clin. Med.*, 1931, **16**, 382 (simplified).

† KREBS and HENSELEIT. *Klin. Woch.*, 1932, **11**, 757, 1137.

most usually associated with oxidation.* The primary product is therefore an α-keto-acid. Thus from alanine the body would produce pyruvic acid :

$$\underset{\text{Alanine}}{CH_3.CHNH_2.COOH} + O = NH_3 + \underset{\text{Pyruvic acid}}{CH_3.CO.COOH}$$

This mode of deamination has been demonstrated to occur with slices of liver, or kidney, suspended in solutions containing various amino-acids and exposed to air,† keto-acids and ammonia being produced. These keto-acids afterwards undergo reduction to a hydroxy-acid, or oxidation to a lower fatty acid, though the conditions which determine whether oxidation or reduction shall take place have not yet been fully studied.

Dakin and Dudley ‡ have shown that under appropriate conditions (removal of the aldehyde as an insoluble compound) alanine may break up in watery solution into pyruvic aldehyde (methyl-glyoxal) and ammonia, thus :

$$CH_3.CHNH_2.COOH = CH_3.CO.CHO + NH_3$$

and this may be a preliminary to the formation of pyruvic acid.

The loss of nitrogen from the molecule diminishes little, if at all, the energy value of the amino-acids of the body. The following Table shows the heat equivalents per gram molecule of two of the amino-acids and their corresponding fatty- and hydroxy-acids :

Substance	Calories per grm. molecule
Leucine	855
Isobutylacetic acid	837
Alanine	389
Propionic acid	367
Lactic acid	329
Pyruvic acid	279

These heat equivalents represent the heat evolved on the total oxidation of the substances in question. In the case of the amino-acids, part of the molecule is not oxidised, the nitrogen leaving the body not as free nitrogen but as urea. To obtain the total possible heat value of an amino-acid to the body, we must subtract from its molar heat equivalent half the heat equivalent of urea, since one molecule of urea is produced from 2 molecules of an amino-acid. The heat equivalent of urea being 80, the physiological heat equivalent of alanine will be 349, as against 329 for lactic acid. Thus even in the case of the smallest molecule, the loss of energy attendant on simple deamination and conversion into the corresponding hydroxy-acid amounts to little more than 5 per cent., and the proportion will be much smaller in the case of the larger molecules.

Although the urea excretion gives an index to protein breakdown, it only shows the extent of the deamination of the protein constituents, and it tells us nothing whatever about the fate of that non-nitrogenous part, which contains 95 per cent. or more of the total energy of the protein food. The rapid excretion of urea after a protein meal is a sign that the body, after satisfying its modest needs for the repair of its tissue waste, has no need for the rest of the nitrogen, and that this is got rid of before the really valuable energy-giving part of the protein molecule is admitted into the metabolic affairs of the cells.

The important problem in the *energy* metabolism of protein is thus the fate and nature of the substances that are left after deamination. We have seen that protein exerts a high specific dynamic action (30 per cent.). Lusk showed that this specific dynamic action of protein is possessed also by certain of the amino-acids. The action is not due to oxidation of the

* NEUBAUER, *Z. f. physiol. Chem.*, 1910, **67**, 230 ; **70**, 1, 326 ; KNOOP, *Z. f. physiol. Chem.*, 1910, **67**, 489 ; KNOOP and KERTESS, *ibid.*, 1911, **71**, 252.

† KREBS. *Z. f. physiol. Chem.*, 1933, **217**, 191 ; **218**, 157.

‡ DAKIN and DUDLEY. *J. Biol. Chem.*, 1913, **14**, 155, 423 **15**, 127, 463 ; **16**, 505 1914, **18**, 29, 91.

amino-acid, as was shown by giving glycine to an animal which had been rendered diabetic by the injection of phlorrhizin. Under these circumstances glycine is converted quantitatively into glucose, which is excreted in the urine, so that the CHO moiety of the glycine molecule undergoes no oxidation in the body. Notwithstanding this fact, glycine produces the same augmentation of metabolism in the phlorrhizinised animal as it would in a normal animal. According to one theory, the specific dynamic action of protein is due to the quality possessed by certain of the amino-acids of stimulating the cells of the body and raising their rate of metabolism. But it cannot be the amino-acids themselves that are the stimulants. This is shown by the fact that, when amino-acids are built up to form new tissues, as in the baby or in the animal recovering from starvation, they exert no specific dynamic action. This action occurs only *when the amino-acids undergo conversion to urea*, and, as might be expected, remains in abeyance after removal of the liver.

The interesting fact that ammonium salts also cause an increased heat production,* and are also converted to urea in the liver, lends further support to the opinion that the specific dynamic action represents heat set free in connection with this conversion.

The molecule which remains after removal of the amino group is, as we have shown, probably an α-ketonic acid : this is probably then converted, by loss of carbon dioxide, and subsequent oxidation, into a fatty acid with one atom of carbon less than the amino-acid :

$$R.CHNH_2COOH \longrightarrow R.CO.COOH \longrightarrow R.COH \longrightarrow R.COOH.$$

or else, by reduction, to a hydroxy acid

$$R.CO.COOH \longrightarrow RCHOH.COOH.$$

The immediate fate of these fatty acids differs according to their nature, though their ultimate fate is complete oxidation to CO_2 and water. It will be clear, as we shall see in studying fat and carbohydrate metabolism, that there will be much in common between their fate and that of fatty acids derived from other sources. Most of our knowledge of these intermediate products is derived from the study of pathological conditions, in which they cannot be further oxidised, or by artificially converting the fatty acids into derivatives, *e.g.* phenyl derivatives, which can be subsequently traced. We shall deal with the intermediate metabolism of the fatty acids later ; in the meantime we may state that the amino-acids are divisible into three groups, according as they form, as intermediate compounds, sugar, aceto-acetic acid, or neither of these substances. Thus, of the amino-acids formed by the digestion of proteins, glycine, alanine, serine, cysteine, aspartic acid, glutamic acid, hydroxy-glutamic acid, ornithine, arginine, proline and hydroxy-proline can under appropriate circumstances be converted quantitatively into glucose. Probably those residues which ultimately form glucose are converted first into pyruvic or lactic acid, from which glucose is readily formed in the liver. Since the formation of glucose reduces the formation or appearance of ketone bodies, these amino-acids are classed as *antiketogenic* amino-acids. On the other hand, leucine, isoleucine, phenyl alanine and tyrosine yield no glucose, even in the diabetic animal ; but may in the liver undergo conversion into aceto-acetic acid, which is a stage in the oxidative disintegration of fats. This group is therefore classed as *ketogenic* amino-acids. Tryptophan, valine, lysine and histidine do not form either product.

* LUNDSGAARD. *Skand. Arch. f. Physiol.*, 1931, **62**, 243.

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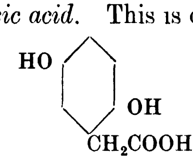
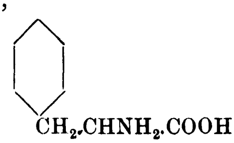
MANN and MAGATH (1924). Die Wirkungen der totalen Leberextirpation. *Ergebn. d. Physiol.*, **23**, 212.

The Fate of the Cyclic Groups in the Protein Molecule. So far, we are acquainted with three compounds of the aromatic series among the products of disintegration of the protein molecule. These are tyrosine, phenyl alanine and tryptophan. Since these substances are also contained in protein constituents of the tissues, we may assume that, after they have been set free and absorbed they are, in part at least, built up again into tissue proteins. Ultimately they can all be completely destroyed, with the production of urea, carbon dioxide and water. In this respect they present a contrast to almost all other compounds of the aromatic series. Thus benzoic acid, benzylalcohol and phenyl propionic acid, when administered, are passed in the urine as hippuric acid (benzoyl glycine). But the ketonic acids corresponding to tyrosine or phenyl alanine (*p*-hydroxy phenyl-pyruvic acid or phenyl pyruvic acid) when given to phlorrhizinised animals yield aceto-acetic acid.

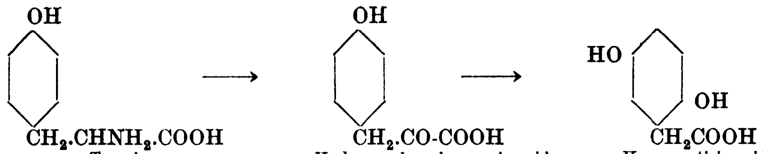
Some light is thrown on the conditions of breakdown of these aromatic bodies by the study of a rare disorder in metabolism, which may occur in certain families and is known as *alcaptonuria*. In this condition, which is congenital and lasts throughout life, the urine darkens considerably when made alkaline and exposed to the air. It has the power of reducing Fehling's solution, so that the presence of sugar might be suspected. On analysis the peculiarities of the urine are found to be due to the presence in it of a substance known as *homogentisic acid*. This is dihydroxyphenyl acetic acid.



The amount of this substance in the urine bears a constant ratio to the nitrogen excreted. If tyrosine or phenyl alanine be administered to patients affected with this disorder, both substances are quantitatively converted into homogentisic acid. The ratio of this acid to the total nitrogen indicates that the whole of the tyrosine and phenyl alanine of the protein molecule, whether set free in the alimentary canal or in the tissue metabolism, is converted into homogentisic acid. The direct conversion of phenylalanine or tyrosine into homogentisic acid seems improbable. Embden and Baldes believed that phenylalanine,



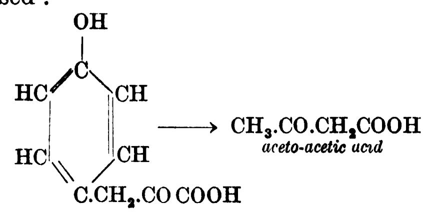
is first oxidised to tyrosine, and that, since phenyl pyruvic acid is also converted in alcaptonuric patients to homogentisic acid (instead of into aceto-acetic acid as it presumably is in the normal), it has been suggested that this, or its *p*-hydroxy derivative, forms a stage in the conversion of tyrosine into homogentisic acid. Thus:—



Tyrosine — *p*-Hydroxy-phenyl-pyruvic acid — Homogentisic acid

It is possible that under normal circumstances tyrosine and phenyl alanine are oxidised to homogentisic acid, as in the alcaptonuric patient. In the normal individual, however, the introduction of two hydroxyl groups into the benzene ring leads to some process, perhaps of an enzyme character, which breaks up the ring. This enzyme is absent in the alcaptonuric, so that the transformation of the phenyl derivatives stops short at the stage of homogentisic acid. The eminently specific character of this process is shown by the fact that a slight modification in the series of conversions renders further change impossible. Thus phenyl acetic acid is incapable of undergoing further oxidation.

In the opinion of Dakin,* however, the formation of homogentisic acid from tyrosine is abnormal, and he considers that normally the benzene ring is split immediately after the formation of hydroxy-phenyl pyruvic acid, the side chain and parts of the ring forming aceto-acetic acid, while the rest of the ring is oxidised :



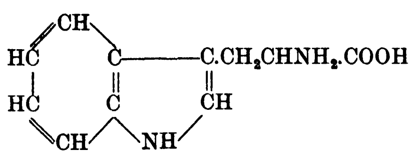
p-hydroxyphenyl-pyruvic acid

In another pathological condition (*Tyrosinosis*) (only once reported), the excreted product was *p*-hydroxyphenyl pyruvic acid, and homogentisic acid if given was destroyed as it is by normal subjects. This supports Dakin's view.

Tyrosine and phenyl alanine are not only constituents of many proteins, but are also starting points in the body for the formation of two important hormones, *adrenaline* and *thyroxine.* Adrenaline is possibly formed by the action of appropriate enzymes, tyramine being an intermediate stage. Tyrosine, when oxidised by the oxidase called tyrosinase, is converted ultimately into a black pigment called *melanin,* which gives the dark colour to hair and skin.

Tyrosine in the intestine may undergo deamination by the action of bacteria, to form hydroxyphenyl propionic acid and hydroxyphenyl acetic acid. These cannot be further oxidised, but appear in the urine as such or, after conversion into cresol or phenol, as sulphuric acid esters.

Somewhat similar conditions apply to the oxidation of tryptophan.† This body is an indol derivative and its formula is :—



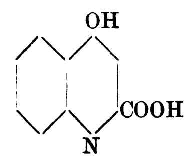
* DAKIN. *J. Biol. Chem.*, 1911, **9**, 151.

† KOTAKE. *Ergebn. d. Physiol.*, 1935 **37**, 245.

i.e. it is indol amino-propionic acid. It undergoes, like tyrosine, complete oxidation in the body, being probably first converted to the corresponding deaminated ketonic acid, indole-pyruvic acid. On the other hand, a very slight alteration in the molecule renders it incapable of this change. Thus some of the tryptophan set free by the tryptic digestion of proteins may, under the influence of the bacteria of the intestine, undergo deamination and reduction with the production of indol propionic acid, and this by oxidation may be converted to indol acetic acid. The latter substance by decarboxylation may be converted into skatol or, by oxidation and further loss of carbon dioxide, into indol. Of these products of bacterial change, indol acetic acid may be found in the urine, and indol and skatol are oxidised to the corresponding phenols and pass into the urine conjugated either with sulphuric acid to form 'urinary indican,' or with glucuronic acid.

Apart from these putrefactive changes due to bacteria, no indol derivatives pass into the urine. The amount of the indol and skatol esters serves therefore as an index of bacterial decomposition in the alimentary canal, but gives no clue to the total tryptophan metabolism of the body. If putrefaction be prevented by the administration of calomel or other intestinal antiseptic, these esters may entirely disappear from the urine. On the other hand, the partial obstruction to the onward passage of food, caused by dividing the small intestine in two places a few inches apart and replacing the intervening length of intestine the wrong way round, increases the indican excretion twenty or thirty-fold. Subcutaneous injection of tryptophan in rabbits does not increase the indoxyl and skatoxyl sulphates ('urinary indican') in the urine, whereas a considerable increase is brought about by subcutaneous injection of indol.

In the dog, tryptophan is converted into *kynurenic acid*, a quinoline derivative



which is excreted in the urine,* but this is probably not a stage in its oxidation in man.

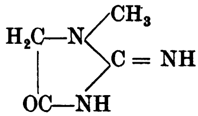
THE PYRROL RING, which occurs in proteins as proline and hydroxyproline (*i.e.* pyrrolidine carboxylic acid and hydroxypyrrolidine carboxylic acid) appears to undergo complete disintegration in the body, three of the C. atoms of each molecule of the deaminated residue being finally converted into glucose. Ornithine or glutamic acid are possible intermediary compounds.

Another ring grouping, iminazol, occurs in histidine, which is iminazol α-amino-propionic acid. This, too, undergoes complete oxidation in the human body. In dogs, administration of histidine leads to excretion of urocanic acid in the urine. This is perhaps formed from histidine by the action of bacteria in the intestine.† It is important to bear in mind that this ring may be produced synthetically by very simple means, *e.g.* by the action of zinc oxide and ammonia on glucose, which results in a rich yield of

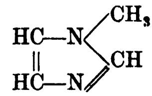
* ELLINGER, *Z. f. Physiol. Chem.*, 1904, **43**, 325; ELLINGER and MATSUOKA, *Z. f. Physiol. Chem.*, 1920, **109**, 259.

† RAISTICK. *Bioch. J.*, 1917, **11**, 71.

methyl iminazol. A similar grouping is found in creatinine, as is seen by comparing the formulæ:



Creatinine



Methyl iminazol

The iminazol group is at present chiefly interesting in that it contributes to the formation of the complex ring compounds known as the purines. Since the purine metabolism is closely connected with the question of the origin of uric acid, we may consider these questions together.

The Excretion of Ammonia. A large proportion of the urea appearing in the urine after a protein meal is exogenous, and is derived from the proteins or their disintegration products almost immediately after their absorption. A certain small proportion of the nitrogen in the urine is generally turned out in the form of ammonia. This proportion is not increased by the administration of ammonium carbonate. If ammonium chloride be given to a starving rabbit, it appears in the urine unchanged, and so increases the proportion of ammonia in this fluid. If, however, the ammonium chloride be administered with sodium bicarbonate or with the ordinary vegetable diet, there is no increase of the ammonia in the urine, the whole of the ammonium chloride being converted into urea. The factor which determines the proportion of ammonia in the urine is the relative proportion of acids and bases which have to be eliminated from the body. The normal acid reaction of urine is due to the presence of such substances as acid sodium phosphate. If the fixed alkalies in the food are sufficient to combine with the whole of the acids excreted from the body, then the urine is neutral and the ammonia will be completely converted into urea and eliminated as such. If, however, a dose of mineral acid be administered to an animal, this must be excreted in combination with some of the base from phosphates ; the urine will then be acid, owing to a preponderance of monobasic phosphate. Some of the acid will also be excreted in combination with ammonia. The ammonia of the urine is therefore an index to the amount of acids which are excreted. These acids may be introduced directly with the food, as when mineral acids are administered by the mouth, or may be the product of abnormal metabolic processes occurring in the body. Thus under certain circumstances, *e.g.* in complete carbohydrate starvation, hydroxy-butyric acid and aceto-acetic acid are produced in the body in large quantities, but cannot undergo further disintegration. The maintenance of a normal concentration of hydrogen ions (neutrality) in the fluid media of the body is a necessary condition for the continuance of life. It is therefore essential that the acids thus formed should be neutralised, carried to the kidneys, and excreted by them in combination with some base. When the alkalies of the food and of the tissues do not suffice for their neutralisation, ammonia, which is a constant intermediate stage in the production of urea, is then utilised for this purpose and the acids appear in the urine in combination with ammonia. The ammonia of the urine therefore gives valuable information as to the formation of acids in abnormal quantities during the processes of metabolism.

Creatinine and Creatine. On comparing the output of the various nitrogenous excreta given in Folin's Table quoted above (p. 965), we see that on a low protein diet the only substance which does not undergo simultaneous diminution is the creatinine. The conclusion at once suggests itself that creatinine might be regarded as an index of the tissue metabolism of protein.

Creatinine has the formula :

$$\begin{array}{l} NH = \begin{array}{l} C.N(CH_3).CH_2 \\ \;| \qquad\qquad\quad | \\ NH \text{———} CO \end{array} \end{array}$$

and is derived by dehydration from creatine (methyl guanidine acetic acid).

$$\begin{array}{l} NH = C.N(CH_3).CH_2COOH \\ \qquad\;\; | \\ \qquad\; NH_2 \end{array}$$

Both are said to be present in the blood, creatine to the extent of about 3–5 mg./100 c.c., mostly present in the red cells, and 'creatinine' about 2 mg./100 c.c. Most of the apparent creatinine of blood, however, is some other, unidentified, substance Muscle contains about 10 mg. creatinine and about 200 mg. creatine (as creatine phosphate) per 100 grammes, so that the body of a man contains about 100 g. of this substance. How or where creatine is formed in the body is uncertain, though possible precursors are arginine, and especially glycine and, more immediately, guanidine-acetic acid.* Its most probable site of formation is in the muscles. In progressive muscular dystrophy, in which creatine is always present in the urine, the creatine excretion is increased by the administration of either substance.† Creatine is also excreted by many normal persons after administration of glycine.‡

Creatinine is certainly to be regarded as a waste product. About 1–1·5 grammes per day is excreted. When given *per os* it is excreted in the urine. The question at issue is whether it is to be regarded as an invariable end-product of the metabolism of tissue protein, or whether it represents the terminal phase of a metabolic process quite apart. There is much accumulated evidence that the urinary creatinine is derived from creatine. But creatinine is excreted in considerable quantities even when a creatine-free diet is given. It has been found, moreover, that creatine administered in moderate amount by the mouth disappears in the body. It would seem probable that this is due to its conversion into creatine phosphate, which is stored in the muscles, of which it forms an invariable and essential constituent. If a large amount be given, creatine appears as such in the urine. In most cases also a certain small proportion is excreted in the form of creatinine.§ After repeated administration of creatine by mouth, steadily increasing amounts of creatine and creatinine are excreted in the urine.‖ This would make it appear that excretion only occurs when the creatine store of the body exceeds a certain limit : but even then not all the creatine is accounted for, so that there appears to be some other fate of creatine than excretion in either form. For instance, it may be stored for a long time in the muscle, or it may be destroyed in some way in the body.

Creatine is normally present in the urine of children before puberty,¶ and of women, especially in pregnancy, but not in that of normal adult men, except when they are starved, or deprived of carbohydrate. It also appears in hyperthyroidism and in various diseases, *e.g.* progressive muscular dystrophy and myasthenia gravis associated with muscular atrophy or weakness. Probably in most cases the excretion is due to failure of storage.

With creatinine, which may be regarded as a waste product, it is different :

* Bodansky. *J. Biol. Chem.*, 1936, **115**, 641.

† Brand *et al.* *Amer. J. Physiol.*, 1929, **90**, 296 , *J. Biol. Chem.*, 1930, **87**, ix., 1932, **92**, lix.; Beard and Barnes, *J. Biol. Chem.*, 1932, **94**, 49.

‡ Adams, Power and Boothby. *Amer. J. Physiol.*, 1935, **111**, 596.

§ Rose and Dimmitt. *J. Biol. Chem.*, 1916, **26**, 345.

‖ Benedict and Osterberg. *J. Biol. Chem.*, 1923, **56** 229.

¶ Rose. *J. Biol. Chem.*, 1911, **10**, 265.

once this substance is formed it is promptly excreted. The amount formed certainly bears some relation to the amount of muscle present, though apparently not to the amount of muscular work done, when long periods are considered. Thus a muscular man may excrete up to 30 mg. creatinine per kg. body-weight per day (creatinine coefficient), whereas in women it is usually about 18 mg. and in children about 15 mg.

The excretion of creatinine was investigated under various conditions by van Hoogenhuyze and Verploegh, and support the view expressed above as to the intimate relation of creatinine with the tissue metabolism. During and immediately after muscular exercise, there is an accelerated output of creatinine in the urine, but there is a corresponding drop during recovery, so that the daily excretion is but little affected. Since we know that during muscular contraction there is a conversion of creatine phosphate into creatine and inorganic phosphate, both of which are diffusible, it is reasonable to suppose that some of the creatine escapes into the general circulation, is converted into creatinine, and excreted as such. During recovery it is possible that the process may be reversed, or at all events checked, as creatine phosphate is re-formed.

The close relation between creatine excretion and carbohydrate depletion on the one hand, and between creatine phosphate and glycogen metabolism on the other hand, seems to suggest a closer immediate relation of creatine metabolism to carbohydrate than to protein metabolism. That the creatine ultimately arises from protein is extremely probable, though by what intermediary stages it is impossible to say. Any small amount of creatine which escapes from its proper combination is converted into creatinine, while larger amounts are excreted unchanged. The greater the mass of muscle in the body, or the greater its rate of destruction, the greater will be this liberation of creatinine.

Other Tissue Products. During protein starvation the uric acid output, though diminished, does not show a change which is at all proportional to that shown by the urea. This substance therefore might also represent an end product of tissue metabolism. Since, however, uric acid is an outcome of the metabolism of a special group of bodies, the nucleins and purine bases, we shall have to devote a complete section to its consideration.

Although the urea is diminished in protein starvation, it still remains the most abundant nitrogenous constituent of the urine. We are therefore not justified in excluding this substance from the products of tissue metabolism. We shall see later that uric acid may possibly also undergo further oxidation with the formation of urea. Even during complete protein starvation, some of the urea which is turned out may be the expression of a utilisation of protein, through deamination, for the energy needs of the body.

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The Fate of Protein Sulphur. The normal source of sulphur is in the cystine and methionine links in proteins. Its fate is by excretion in bile, as the taurine of taurocholic acid, by shedding of hair and nails (as keratin) and by excretion in the urine. Sulphur occurs in the urine in three forms: as inorganic sulphates, as ethereal sulphates (indoxyl- and skatoxyl-sulphates), and in an unoxidised condition often termed neutral sulphur. This last consists of cystine, thiocyanates, and, in some animals, mercaptan compounds. The excretion of the inorganic sulphates rises *pari passu* with

that of the urea so that, with the throwing off of the NH_2 group, there must be also a removal of the greater part of the sulphur of the protein molecule. The sulphur does not necessarily appear in the urine simultaneously with the nitrogen derived from the same protein. If, for instance, there is a positive N-balance, *i.e.* if protein is being built up more rapidly than broken down, the excretion of S lags behind, and in the reverse condition of excessive N-catabolism, the N lags behind.

The ethereal sulphates are excreted in varying quantity, according to the extent of the decomposition processes which are occurring in the intestine, whereby the tryptophan, produced in the digestion of proteins, is converted into indol and skatol. These two substances, after absorption, are conjugated with sulphuric acid to form the indoxyl- and skatoxyl-sulphates of the urine. If the processes of putrefaction are increased, as in intestinal obstruction, the relative amount of sulphate appearing in the conjugated form is also increased. On administration of phenol, a large proportion of the sulphate appears in the urine conjugated with phenol or with products of its oxidation. If the normal putrefactive processes which go on in the intestine are abolished by the administration of intestinal antiseptics, the ethereal sulphates practically disappear from the urine. We cannot therefore regard the absence or diminution of the ethereal sulphates during protein starvation as throwing any light on the endogenous protein metabolism. On the other hand, the fact that the neutral sulphur undergoes no decrease suggests that this part of the sulphur output of the organism may be connected with tissue metabolism. Further observations on the output of neutral sulphur during fever or wasting diseases are necessary before a definite conclusion can be arrived at on this point. Neutral sulphur excretion amounts to about 100 mg. daily, but varies widely ; about 8 per cent. of it is in the form of cystine, of which an average of about 30 mg. is excreted daily.*

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2. NUCLEIN METABOLISM

An outline of the chemistry of nucleoproteins has already been given in Chapter IV.

The nucleoproteins are especially abundant constituents of nuclei, and therefore occur to a greater or less extent in all the ordinary animal foods, eggs and milk excepted. Other important purine derivatives are the mononucleotides, adenylic acid and guanylic acid, which occur in the cytoplasm of many tissue cells, *e.g.* in muscle, where adenylic acid is present as a pyrophosphate and plays an important part in the metabolism of that tissue. Just as the metabolism of proteins is the metabolism of the amino-acids, so the metabolism of the nucleoproteins and nucleotides is essentially comprised in the history of their characteristic constituents, the purines and pyrimi-

* MEDES. *Bioch. J.*, 1937, **31**, 12.

dines. The protein portion of the molecule, which contains a high proportion of diamino acids, shares the common fate of other protein in metabolism.

Formation of Nucleins in the Body. We have seen that, in the higher animals at any rate, there is little power of building up proteins from bodies simpler than their constituent amino-acids. The nucleotides, on the other hand, can certainly be synthesised by the animal. This is shown by the fact that the hen's egg before incubation contains practically no nuclein or purine bases. During incubation there is a rapid increase in the number of nuclei, so that the chick just before it is hatched contains a considerable amount of nuclein from which purine and pyrimidine bases can be extracted. These must have been formed by a synthesis from the phosphoproteins and phosphatides which form so important a constituent of the egg-yolk.* In the same way, in the young infant there is a rapid increase in the nucleoprotein of the body, although the only food ingested is milk, which contains but an insignificant amount of purine compounds. Confirmation of the synthesis of purines in the body has also been given by Benedict: he fed a Dalmatian hound on a purine-free diet, yet was able to extract 100 grammes of uric acid from its urine.† There is no definite evidence as to the steps in this synthesis, though there are indications that arginine and histidine may be starting points.‡ The power of synthesis of purines possessed by the body must complicate the question of their fate after ingestion, since it is evident, either that they can be destroyed and excreted in some other form, or that the products of their destruction may be built up again into fresh purine or nuclein molecules.

Fate of Nucleoproteins in the Body. Nucleoprotein metabolism may be exogenous or endogenous, *i.e.* the nucleoprotein broken down in the body may be derived either from the food or from the tissues. Most of our knowledge on this subject relates to the purine portions of the molecule, the pyrimidine part having been much less explored. We will first deal with the exogenous nucleoprotein.

The changes undergone by ingested plant and animal nucleic acids differ in detail, and only the broadest outlines can be given here. In the stomach, nucleoproteins are acted upon by the pepsin of the gastric juice, the protein being digested away and nuclein remaining. In the duodenum this is attacked by the trypsin of the pancreatic juice, with removal and digestion of the more firmly bound protein part, so that nucleic acid remains. This is not further attacked by pancreatic juice.

The nucleic acids are tetranucleotides, in which four mononucleotides are linked together. Thus. a tetranucleotide of animal origin would probably have the structure :—

```
                        Phosphoric acid—Pentose—Guanine
                                           |
                Phosphoric acid—Pentose—Cytosine
                                   |
        Phosphoric acid—Pentose—Thymine
                           |
Phosphoric acid—Pentose—Adenine
```

In the intestine, part of the nucleotides, &c. are destroyed by the action

* PLIMMER and SCOTT. *J. Physiol.*, 1909, **38**, 247.
† BENEDICT. *J. Lab. Clin. Med.*, 1916, **2**, 1.
‡ ACKROYD and HOPKINS. *Bioch. J.*, 1916, **10**, 551.

of bacteria, but there are also further digestive enzymes here which act on them.

The intestinal juice and mucosa contains an enzyme, or enzymes (*poly-nucleotidase*), which splits up the nucleic acid into its four constituent mono-nucleotides,* *e.g.*

Phosphoric acid—Pentose—Adenine (= *Adenylic acid*)

is a purine nucleotide, and

Phosphoric acid—Pentose—Cytosine

is a pyrimidine nucleotide ; it is likely that some at least of these products are absorbed as such, since an adenine nucleotide has been recovered from human blood.† However, further changes can be effected by the intestinal juice, or by the cells of the intestinal mucosa. The latter contain the enzyme *nucleotidase*, a phosphatase,‡ by which the phosphoric acid is split off from the nucleotides, with formation of nucleosides, *e.g.*

Phosphoric acid—Pentose—Adenine ——→ Pentose—Adenine (= *Adenosine*, a nucleoside).

The nucleosides are further acted upon by another enzyme, also present in the intestinal mucosa, and called *nucleosidase* §; this breaks up the purine nucleosides into purine base and carbohydrate, *e.g.* :—

Adenosine
↙ ↘
Carbohydrate (Pentose) Adenine

There may therefore be present for absorption in the intestine, nucleotides, nucleosides, purine bases (guanine and adenine), carbohydrate and phosphoric acid. These products presumably may all enter the blood and be dealt with, together with similar products formed, we know not where or how, in endogenous purine metabolism, which we must now discuss.

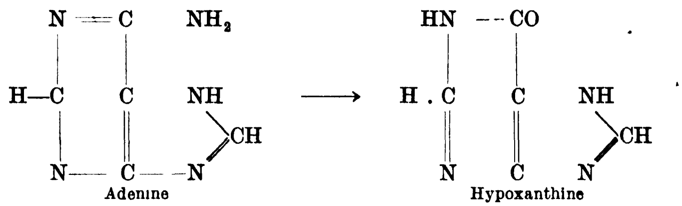
The chief ultimate purine end product excreted in man is uric acid. In some animals it is allantoin. Ingestion of nucleoprotein, nucleic acid or purine bases is, in man, followed by an increased excretion of uric acid in the urine. It is evident that the uric acid of the urine may be derived either from the nucleins, &c. of the food, or from the breakdown of the nucleoproteins of the tissues of the body. By digestion of nucleic acids with animal tissues or their extracts under varying conditions, it is possible to bring about all the changes mentioned above, and also those further ones involved in the conversion of the purine bases contained in them into uric acid. Probably by no means all the purine bodies involved in metabolic changes are excreted as such, a good deal being lost in some unknown way.

Tissue-nucleotidases are of different kinds, phosphonucleotidases splitting off the phosphoric acid and leaving the nucleosides, while the purine nucleotidases which are more effective in a slightly alkaline medium, split off the purines, leaving the phosphoric acid combined with

* Levene and Dillon. *J. Biol. Chem.*, 1932, **96**, 461.
† Jackson. *J. Biol. Chem.*, 1924, **59**, 529.
‡ Levene and Dillon. *J. Biol. Chem.*, 1930, **88**, 753.
§ Levene and Dmochowski *J. Biol. Chem* .1931 **93**, 563

the carbohydrate. (This could then be split into its components by a phosphatase.)

The purines set free in these various ways undergo further changes. One of these is deamination. Thus, guanine and adenine, acted upon by the enzymes guanase and adenase, are converted respectively into xanthine and hypoxanthine by loss of their amino groups, *e.g.*



But deamination can also occur before the purines are liberated, *i.e.* in the nucleosides. Thus adenosine can yield inosine by the action of an appropriate deaminase. By a similar change even the nucleotide can be deaminated, *e.g.* adenylic acid can be converted to inosinic acid, and from this a purine nucleotidase can split off hypoxanthine.

The hypoxanthine is converted under the action of an oxidase (xanthine oxidase) first into xanthine and then into uric acid. This was one of the earliest facts discovered in the metabolism of purines. Horbaczewski * showed that, if spleen pulp be digested with blood for some time, it is possible to extract a considerable amount of xanthine from the mixture. If, however, oxygen be bubbled through the fluid, the xanthine disappears, its place being taken by uric acid. All these changes occur in the living body, though not necessarily in the order just set out. Thus a pulp of dog's liver is able completely to deaminise the adenine group of nucleic acid, converting it into a hypoxanthine group ; but it is without effect on free adenine. It is evident, therefore, that the various enzymes which have been described act partly on the whole nucleotide molecule, partly on the products of its decomposition, and that the results of the action of the body enzymes are not the same in the two cases.

These various enzymes are not all present in all tissues, nor are the same ones present in the same tissues of different animals. Thus human liver contains guanase and xanthine oxidase ; guanase is present in one or other tissue of most animals, except in those of the pig. Adenase is absent from human tissues † ; guanase is present in human kidney, liver and lung, but not in spleen or pancreas. Xanthine oxidase is present in the human liver but not in other human tissues. For these reasons there are species differences in the way in which the purines are dealt with ; for instance, the muscles of the pig often contain deposits of guanine because this cannot be destroyed, while adenine is present in human urine in small amounts. Yet not all the adenine present in the compounds ingested is excreted in man, because much of this purine is deaminised, by enzymes other than adenase, while still a part of the nucleotide or nucleoside molecule. ‡

If we make this reservation that the constituent parts of the nucleic acid molecule may undergo changes while still bound to the other parts, we may represent diagrammatically the formation of uric acid from nucleic acid as follows :—

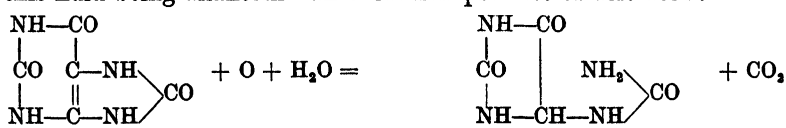
* Horbaczewski. *Monat. f. Chem.*, 1891, **12**, 221.
† Jones. *J. Biol. Chem.*, 1911, **9**, 129, 169.
‡ Jones and Winternitz. *Z. f. Physiol. Chem.*, 1905, **44**, 1 ; 1909, **60**, 180.

Enzymes	
	Nucleic acid
Phospho Nucleotidase	Phosphoric acid; Purine nucleosides; Pyrimidine nucleosides
	Purine nucleosides → Guanosine, Adenosine; Pyrimidine nucleosides → Uridine, Cytidine (Fate unknown)
Deaminase	Guanosine → Xanthosine; Adenosine → Inosine
Hydrolysis	Xanthosine → Xanthine; Inosine → Hypoxanthine
Oxidase	Hypoxanthine → Xanthine
Oxidase	Xanthine → Uric acid
Oxidase (*Uricase*)	Uric acid → Allantoin (in dogs)

or

	Nucleic acid
Nucleicacidase	4 Mononucleotides
Purine nucleotidase	Pentose-phosphoric acid; Guanine; Adenine; Pyrimidine base
Deaminase (Guanase and adenase)	Guanine → Xanthine; Adenine → Hypoxanthine
Oxidase	Hypoxanthine → Xanthine
Oxidase	Xanthine → Uric acid

The question arises whether the uric acid excreted by a man represents the whole of the nucleins which have been destroyed in the body. Although complete equivalence has been found between the amount of hypoxanthine ingested and the amount of uric acid excreted, the same equivalence has not been established in the case of nucleic acid; and the important question arises whether uric acid once formed is stable or whether it may undergo further changes before being excreted. In many animals, such as the dog, the amount of uric acid in the urine is only minute, the chief purine derivative in this fluid being allantoin—an oxidation product of uric acid:



This transformation can be effected by extracts made from the liver of the dog and various other animals.* The enzyme carrying out this change is known as uricase. No such enzyme is found in any human organs.†

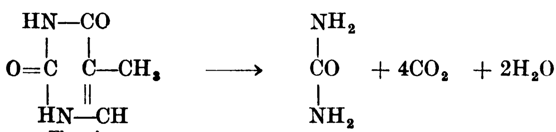
If we add together the amounts of allantoin and uric acid excreted, and express the allantoin as a percentage of this sum, we get a figure called the *uricolytic index*. It is a measure of the extent to which uric acid is converted in the body into allantoin (by the enzyme uricase). In dogs of most species it is 98, *i.e.* most of the uric acid is oxidised to allantoin before excretion, but in the Dalmatian hound it is only 32. In the chimpanzee it is 0, and in man only 2. In man and the higher apes, therefore, practically all the uric

* Wiechowski u. Wiener. *Hofmeister's Beitr.*, 1907, **9**, 247, 295.
† Jones. *Z. f. Physiol. Chem.*, 1909, **60**, 180.

acid formed in the body would appear to be excreted as such in the urine. The small trace of allantoin which may occur in human urine is directly derived from the food.

On the other hand, it is important to bear in mind the possibility that some of the uric acid which occurs in human urine may arise by a process of synthesis. In the bird the greater part of the uric acid is derived not from purines at all, but by a process of synthesis from lactic acid and ammonia; and though we have no evidence of a similar change occurring in the mammal, we are not able definitely to exclude its possibility.

So far we have not considered the fate of the pyrimidine portion of the nucleotide molecule. It would seem that the human body, and, in most circumstances that of most other animals, is able completely to destroy these compounds, since they are absent from the urine. If a large amount of thymine or uracil is administered to dogs, some of it passes out in the urine unchanged, though, if given slowly it can be destroyed so that none is recoverable from the urine. Since in this condition the output of urea is increased, it would appear that the pyrimidines are converted into urea. Though we have no knowledge of the site or mode of this transformation, it is, chemically speaking, a simple one, as shown:



Thymine

EXCRETION OF URIC ACID. Normal human blood contains 2 to 3 mg. of uric acid per 100 c.c., but the bloods of animals which do not excrete it contain very small amounts. When given intravenously, as lithium urate, to man, a variable fraction is excreted during the next few days; the kidney does not seem able to deal with it at all rapidly. Under ordinary circumstances a man excretes about half a gramme of uric acid per day. In addition the urine contains a small amount of purine bases. From 10,000 litres of human urine Krüger and Salomon * succeeded in isolating xanthine (10·1 grams), hypoxanthine (8·5 grams), adenine (3·5 grams). These are probably derived from the uric acid metabolism. In addition there are various methylated purines, derived from such constituents of the diet as caffeine, &c.

An attempt has been made to arrive at the amount of uric acid produced endogenously from a study of the quantity of uric acid in the urine of subjects on different diets.

During starvation, when a man is living on his own tissues, one might expect the uric acid to be increased in consequence of disintegration of the tissues. It has been suggested that the amount of the endogenous uric acid in the urine would be obtained by an analysis of the urine from subjects taking a diet free from purine bases, but containing sufficient nitrogen to maintain nitrogenous equilibrium. Even in these circumstances, however, the amount of uric acid increases with the total nitrogenous metabolism, and, in fact, increases after each meal (Fig. 568).

There are three conditions which give rise to a definite increase in the output of endogenous uric acid. These are (1) severe muscular activity, (2) febrile states accompanied by increased nitrogenous metabolism. In both these conditions we may regard the uric acid as derived especially from the adenylic acid contained in the muscle. (3) In maturation of red cells,

* KRÜGER and SALOMON. *Z. f. Physiol. Chem.*, 1898, **24**, 364; **26**, 350.

as, *e.g.* when a pernicious anæmia subject has an induced reticulocyte response resulting from the administration of ventriculin. Presumably the increased uric acid is derived from the destroyed nuclei of the erythroblasts.

The foods which are especially effective in causing increase in the exogenous uric acid are those rich in nuclein, such as sweetbreads or liver, and those rich in hypoxanthine or its precursors, such as meat or meat extract.

When these foods are taken, or when nucleic acid itself is administered, a condition of leucocytosis is generally produced, the number of leucocytes in the blood being increased as much as three times. It has even been suggested that the uric acid is actually derived from a disintegration of the newly formed leucocytes and not by a direct

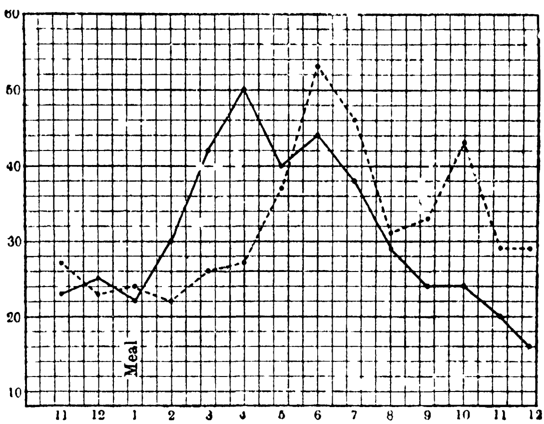


FIG. 568. Curves showing the Hourly Excretion of Uric Acid and Urea after a Single Meal (HOPKINS and HOPE J. Physiol., 1898, **23,** 271)
The continuous line = uric acid output; the dotted line = urea output.

conversion of the purines of the food. But the absence of any absolute proportionality between the degree of leucocytosis and the amount of uric acid excreted points to the probability of a direct conversion of the purines of the food into uric acid.

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3. FAT METABOLISM

The study of fat metabolism entails that of the metabolism of all compounds which contain fatty acids, and we shall begin therefore with a consideration of depôt fat.

DEPÔT FAT

We have seen that the amount of fat in the body is the main factor governing the length of life during starvation, because it is then the only material which can serve as a source of energy for a sufficient length of

time. This reserve material, which is chiefly neutral fat, is found widely distributed in the body and in greatly varying amounts; the body of the average person contains some 12 per cent. of its total weight as fat, nearly all of which can act as an energy reserve. Its distribution in the body varies considerably, but we may say that the *panniculus adiposus* under the skin may account for, roughly, 50 per cent., and the perirenal, mesenteric and omental fat for about 15 per cent., 10 and 5 per cent. respectively, while some 5 per cent. or more may occur in the intermuscular connective tissue. In this adipose tissue the fat is enclosed within distended connective tissue cells, the protoplasm of which is reduced to a thin pellicle round each fat globule.

Composition of Depôt Fat

This reserve fat occurs in the form of triglycerides of various fatty acids the chief of which are stearic, palmitic and oleic acids. Ordinary depôt fat contains 75 per cent. of oleic acid, with 20 per cent. of palmitic acid and 5 per cent. of stearic acid, combined as mixed triglycerides, although often small amounts of more unsaturated acids also occur. There are also present small amounts of lecithin and cholesterol, which we may ignore for the moment.

The degree of saturation of depôt fat depends on its position in the body, there being a decreasing iodine value from the surface inwards. Thus, in a pig fed on barley the iodine values of the subcutaneous fat at increasing distances from the surface were 60, 57, 52 and 51 respectively; this is to be correlated with the increase of temperature as the distance from the skin increases,* and depends in part on the fact that the more unsaturated fats are liquid at lower temperatures than are the saturated ones. The same phenomenon is shown in the fact that the subcutaneous fat of the new-born infant has an iodine value of 47, which increases during the first year to the adult figure of 70.† This again is to be correlated with the internal position of the fœtus and the gradual adaptation during the first year of life to the fall in temperature of the subcutaneous tissues.

The Origin and Composition of Depôt Fat

(*a*) *The Food Fat.* It is a familiar experience that the fats of different species of animals usually have characteristic appearances, *e.g.* the hard fat of mutton, compared with the soft fat of beef. This relative constancy of composition within a species depends to a considerable extent on the similarity of the food of its members. But food fat may also undergo some modification during assimilation, which again will tend to make the fat of a particular animal of a constant nature. Although the nature of the depôt fat is capable of modification by the provision in the food of fats unusual in amount or nature, profound changes in its nature are usually only observed in experimental conditions so severe as to be unphysiological. The most graphic method of demonstrating such changes is to starve an animal until its fat reserves have been largely exhausted and then to fatten it on a particular fat; it is then found that the depôt fat approximates closely in composition to that of the fat given. In experiments of this type linseed oil caused in dogs an adipose tissue containing fat which was liquid at 0° C., while mutton fat resulted in a depôt fat having a melting point greater than 50° C.‡ Similarly, abnormal acids such as erucic acid have

* Henriques and Hansen. *Skand. Arch. f. Physiol.*, 1901, **11**, 151.
† Channon and Harrison. *Biochem. J.*, 1926, **20**, 84.
‡ Lebedeff. *Pflüger's Arch.*, 1883, **31**, 11.

appeared in depôt fat as a result of feeding oils containing it under similar conditions.* Another way of showing changes of this nature is to feed animals on diets in which fat constitutes a very high proportion of the food energy. The following Table illustrates the length to which this process can be carried.† Rats were fed on a basal diet with the addition of various fats in amounts such that these fats constituted 60 per cent. of the total calorie intake. The iodine value of the food and depôt fats which were determined are recorded in the following Table :—

	Iodine values.	
	Food fat	Depôt fat
Soya bean oil	132	122
Corn oil	124	114
Cotton seed oil	108	107
Lard	63	71
Butter fat	36	55

In more physiological conditions, in which unusual fats are given in normal amounts, the depôt fat usually shows a relatively slight change in composition. Since fat is only deposited when food in excess of the energy requirements is ingested, this is to be expected, for the fat of the food will tend to be used for immediate energy purposes. Usually the lower fatty acids are not deposited in the depôt fat, but are either oxidised immediately or built up into higher fatty acids ; this again illustrates the ability of the animal to maintain the depôt fat reasonably constant in composition.‡

It would be expected that the composition of milk fat would also be markedly affected by the addition of fat to the diet, for a cow may be producing daily several pounds of fat in its milk, while its normal food contains a relatively small amount of fat. Such is the case. In one experiment, when a cow was fed on linseed oil, the iodine number of the milk fat rose from 30, its normal figure, to 70·4. Further, after the introduction of iodised fat subcutaneously, iodised fats are found in the milk. In another experiment a bitch, which had been fed with mutton suet and had deposited in her tissues a fat of high melting point, produced a milk the iodine number of which was the same as that of the mutton suet. In this case the fat of the milk had evidently been derived from the food *viâ* the tissues, since during lactation the animal was being fed on meat which was poor in fat.

This question of the effect of feeding with fat on the composition of depôt and milk fats is one of great importance in stock raising, since food concentrates, often the bye-products of other industries, are much used for fattening purposes. The question of flavour has also to be considered, because particular food fats may readily cause the appearance in the fat, or in the milk, of any flavour present in them.

(*b*) *The Formation of Fat from Carbohydrate.* It has long been known to farmers that animals may be fattened on a diet rich in carbohydrate and relatively poor in fat. The classical experiment demonstrating the conversion of carbohydrate into fat in the animal body was that of Lawes and Gilbert.§ In this experiment one of two pigs taken from the same litter was killed and completely analysed. The other pig received a diet for a given period and the total intake of fat, carbohydrate and protein in the diet was measured. At the end of the experiment this animal also was

* MUNK. *Arch. f. path. Anat. u. Physiol.*, 1884, **95**, 407.
† ANDERSON and MENDEL. *J. Biol. Chem.*, 1928, **76**, 729.
‡ ECKSTEIN. *J. Biol. Chem.*, 1929, **81**, 613 ; POWELL. *J. Biol. Chem.*, 1930, **89**, 43.
§ *Phil. Trans. Roy. Soc.*, 1859 ; *J. Anat. and Phys.*, 1877, **11**, 577.

killed and analysed and a balance sheet drawn up, in which the essential points are as follows :—

Total body fat increase	71·2 lbs.	Protein in food	64 lbs.
Fat in food	12·4 ,,	Body protein increase	6·5 ,,
Fat synthesised	58·8 ,,	Protein available for fat synthesis	57·5 ,,
Carbon in fat synthesised	45·3 ,,	Carbon in 57·5 lb. protein	27·4 ,,

From these figures it is seen that whereas the synthesised fat contained 45·3 lb. of carbon, that available from protein was only 27·4 lb., *i.e.* 17·9 lb. of carbon had appeared as fat and must have been derived from carbohydrate.

Fattening of animals on a high carbohydrate diet causes the production of a harder fat than is normal,* with a corresponding decrease in its iodine value, which illustrates again the influence of food on depôt fat composition.

The means whereby sugar is converted into fatty acids is not known, although it is almost certain that it is not converted to a fatty acid by direct condensation ; an initial breakdown and a resynthesis is the probable path. Since carbohydrate metabolism both in the animal and in alcoholic fermentation passes through the three carbon stages (pyruvic acid or lactic acid), it is very probable that one or other of these two compounds forms the starting point from which fat is synthesised. Aldehydes possess the property of condensing together to form long chain compounds containing secondary alcoholic groups, which may lose water with the production of double bonds.

Various schemes to explain the formation of fatty acid from carbohydrate have been postulated, based on this property. One such is briefly outlined below . †

$$\underset{\textit{pyruvic acid}}{CH_3CO.COOH} \longrightarrow \underset{\textit{acetaldehyde}}{CH_3CHO} + CO_2$$

$$CH_3CHO + CH_3CO.COOH \longrightarrow CH_3CH = CH.CO.COOH + H_2O$$

$$\downarrow$$

$$\underset{\textit{crotonic aldehyde}}{CH_3CH = CH.CHO} + CO_2$$

(A)

$$\downarrow \textit{condensation with pyruvic acid}$$

$$CH_3CH = CH.CH = CH.CO.COOH$$

$$\downarrow -CO_2$$

$$CH_3CH = CH.CH = CH.CHO + CO_2$$

$$\downarrow$$

(B)

$$\downarrow H_2$$

$$\underset{\textit{butyric aldehyde}}{CH_3CH_2CH_2CHO}$$

$$\downarrow O_2$$

$$\underset{\textit{butyric acid}}{CH_3CH_2CH_2COOH}$$

In the above scheme a molecule of acetaldehyde derived from pyruvic acid condenses with a further molecule of pyruvic acid with the production of an unsaturated keto acid, which loses carbon dioxide to yield the unsaturated crotonic aldehyde. This latter by reduction would yield butyric aldehyde, which on oxidation would yield butyric acid (B). Instead of following this path, crotonic acid could condense with a further molecule of pyruvic acid and by the same procedure an unsaturated aldehyde with six carbon atoms is produced (A). This compound could either be reduced and oxidised to produce hexoic acid, or condensed further. By similar processes fatty acids containing any *even number of carbon atoms* could be synthesised and they would be saturated or unsaturated dependent on the extent to which the double bonds took up hydrogen. The fatty acids so produced would then be coupled with glycerol which is, of course, readily formed in the body from glucose, by way of glyceric aldehyde.‡ Such a theory

* ELLIS and ZELLER. *J. Biol. Chem.*, 1930, **8**, 185.

† LUBRZYRSKA and SMEDLEY. *Biochem. J.*, 1913, **7**, 375.

‡ EMBDEN, SCHMITZ and BOLDES. *Biochem. Z.*, 1912, **45**, 174

would satisfy the fact that a whole series of even numbered saturated fatty acids may be produced from carbohydrate, together with unsaturated acids. Oleic acid is quantitatively by far the most important unsaturated acid synthesised from sugar, the amounts of other unsaturated acids being very small.

(*c*) *The Formation of Fat from Protein.* The proteins are absorbed as amino-acids and we know that some 60 per cent. of the amino-acids in the food can be converted to carbohydrate in the body. It might reasonably be thought, therefore, that some of this might be converted to fat. There are, however, two limitations which make it certain that fat does not normally arise from protein. The first of these is that amino-acids can only be converted to glucose after their deamination in the liver. This is a relatively slow process, so that there is never made available at any given moment a large amount of sugar derived from amino-acids; hence, after a high protein meal there is no pronounced rise in blood sugar. Secondly, it is difficult to satisfy the normal energy requirements of the body by ingestion of protein only. Hence any carbohydrate produced from protein would be at once oxidised for immediate energy purposes. In particular conditions, nevertheless, fat may be formed from protein, as was shown by the experiment of Atkinson, Rapport and Lusk,* in which large amounts of protein were given to a well-nourished animal resting in a warm environment. Some lower forms of animal life normally form fat on an exclusively protein diet, as was shown by Weinland for certain fly larvæ.

Utilisation of Depôt Fat

The power of the organism to store up reserve carbohydrate is strictly limited and probably 400 grammes represents the extreme limit of storage of carbohydrates in the body. On the other hand, in most animals there is practically no limit to the amount of fat which can be laid down by overfeeding, since the amount of reserve fat deposited will clearly depend on excess of income over expenditure. As soon as the energy expenditure exceeds the income, this fat store is drawn upon, so that adipose tissue is the one which presents the greatest loss during starvation, during which as much as 97 per cent. of it may disappear.

(*a*) *Functions of Depôt Fat.* As a reserve material, fat possesses very great advantages over carbohydrate or protein. In the first place its calorific value, 9·3, is more than twice as great as that of these other foodstuffs, 4·1. Secondly, the storage of either protein or carbohydrate entails the presence of much water, while adipose tissue is virtually pure fat (95 per cent.). Thus an enormous energy reserve may be laid down in a relatively small space. This fat in the depôts is not a static dump of material, but is constantly being turned over, even when the total amount is unchanged, or increasing. This can be shown by feeding with 'labelled' fats, *e.g.* with fat containing deuterium.

Besides this function as reserve material, fat serves as a protection against temperature change and against mechanical injury, a simple illustration of the former being the apparent immunity of women, as compared with men, to exposure to cold surroundings; an immunity which women in a normal state of nutrition owe to the fact that nature has been more liberal, as art is more tolerant, of subcutaneous fat in the female than in the male.

(*b*) *The General Utilisation of Fats in the Body.* After a few days' starvation the sole sources of the energy which is evolved must be fats

* *J. Biol. Chem.*, 1922, **53**, 155.

and proteins, and it is possible by an estimation of the nitrogen output to determine what fraction of the total energy evolved is to be ascribed to protein metabolism. Thus in the case of Cetti, the professional faster, it was found that the nitrogenous metabolism per unit of body weight between the fifth and tenth days of starvation corresponded to an average of 1 gramme of protein per kilogramme body weight daily. In order to oxidise this amount of protein about 1 c.c. of oxygen per minute would be required. Cetti's total oxygen consumption was 5 c.c. per kilogramme per minute, so that four-fifths of the oxygen absorbed was required for the oxidation of non-nitrogenous substances and these could only have been fats. In animals with a large store of fat the proportion of the energy obtained at the cost of the fats may be still greater.

How the fat leaves the depôts is uncertain, but there is some evidence that it leaves, as it enters, in the form of fine particles of neutral fat in the blood. During fasting these are called '*lipomicrons*'; they show periodic bursts of increase at five-hourly intervals during starvation.

The oxidation of fats supplies energy, not only for the production of heat, but also for the performance of mechanical work. Fat is found as a normal constituent of all muscle fibres and the amount of this substance is greater in proportion to the activity of the muscles concerned. Thus the heart muscle and the red muscle of the diaphragm contain larger amounts of fat than the pale voluntary muscles which have to undertake only short periods of activity.* In the human heart muscle 15 per cent. of the solids are soluble in ether and more than one-half of the ether extract is composed of fat; this would be sufficient to supply the energy of the contracting heart for six or seven hours' work. Evidence from various sources suggests that the heart does use fat to provide energy. In most instances, however, it seems that the tissues use carbohydrate in preference in order to supply the energy.

In prolonged moderate exercise the respiratory quotient is the same as during rest, provided that the diet remains the same. The respiratory quotient under these conditions depends merely on the nature of the food oxidised, and it makes no difference what intermediate changes take place, such as that of fat to carbohydrates or *vice versâ*, so long as all the food used finally undergoes complete oxidation. This is well shown in the following Table, in which the oxygen consumption and respiratory quotient are compared in a man resting and working on three different diets—one principally fat, one principally carbohydrate, and the other principally protein.

Diet principally	Resting		Working		kg m of work done	Per kg m of work	
	c c. oxygen used per min.	Resp. quotient	c c. oxygen used per min.	Resp. quotient		c.c. oxygen used	Cal.
Fat . . .	319	0·72	1029	0·72	354	2·01	9·39
Carbohydrate .	277	0·90	1029	0·90	346	2·17	10·41
Protein . .	306	0·80	1127	0·80	345	2·38	11·35

During short bouts of severe exercise, as we have seen in Chapter X., the respiratory quotient of the *excess* metabolism is about unity, which might be interpreted as meaning that the extra energy is derived by the oxidation of carbohydrate. This is the case even when the subject is on a fat diet. But in that case after the exercise, or if hard exercise is

* LEATHES. *J. Physiol.*, 1904, **31**, Proc. ii.

prolonged, the quotient falls and this is interpreted as indicating that the muscle (and liver) are replenishing their stores of glycogen at the expense of fat.

(*c*) *The Possible Conversion of Fat into Sugar.* When a starved rat is fed on a fatty acid containing an *odd* number of carbon atoms (*e.g.* propionic acid), an accumulation of glycogen is demonstrable in the liver. But natural fats only contain fatty acids of even carbon numbers and do not produce this effect. Yet there is good evidence that constant production of glucose is normally proceeding in the liver, even during fasting, and this glucose must arise from fat.* Further, the perfusion of livers with blood containing butyric acid leads to the production, not only of ketone bodies, but also, it is claimed, of glucose.† Also, the evidence that the energy of muscular contraction is derived largely from carbohydrate requires, as a logical necessity, the possibility, at all events during fasting, of the conversion in the body of fat into carbohydrate.

During hibernation, as Pembrey showed, the marmot has a very low respiratory quotient, which may be as small as 0·3 or 0·4. The excess intake of oxygen can be so great as to cause an increase in the weight of the animal. This retention of oxygen can be explained only by assuming that there is going on in the body a conversion such as that of fats into carbohydrates.

The same conversion, accompanied by a very low respiratory quotient, has been alleged to take place in certain cases of diabetes. In many cases, when the diabetic animal is fasting, or is receiving a purely protein diet, a uniform ratio has been found to exist between the glucose and the nitrogen excreted, *i.e.*

$$\frac{G}{N} \text{ generally equals } 2{\cdot}8$$

In certain other cases a constant G : N ratio of 3·65 has been found. The former represents a conversion into sugar of 45 per cent., the latter of 58 per cent. of the protein which was used. In a few cases, however, even during complete starvation, the G : N ratio has been stated to amount to as much as 10 or 12. These animals are stated to be practically free from carbohydrates, so that the sugar excreted in the urine can come only from the breakdown of fats, since the protein could not provide all of it.

Various suggestions have been made as to the path by which fat could be converted to carbohydrate. For instance ‡:—

$CH_3CH_2CH_2COOH$	butyric acid
$\downarrow (+\ O)$	
$CH_3CHOH.CH_2COOH$	β-hydroxy butyric acid
$\downarrow (+\ O)$	
$CH_3CO.CH_2COOH$	aceto-acetic acid
$\downarrow (+\ H_2O)$	
$CH_3CHOH.CHOH.COOH$	di-hydroxy butyric acid
$\downarrow (-\ H_2)$	
$CH_3C.OH = COH.COOH$	di-hydroxy crotonic acid
$\downarrow (-\ H_2)$	
$CH_3CO.CO.COOH$	diketo butyric acid
$\downarrow (-\ CO_2)$	
$CH_3CO.COH$	methyl glyoxal
$\downarrow (+H_2O)$	
$CH_3CHOH.COOH$	lactic acid
$\downarrow$	
glucose	

* Young. *J. Physiol.*, 1936, **87**, 11 P.; Soskin *et al* *Amer. J. Physiol.*, 1935, **114**, 110.
† Blixenkrone-Møller. *Z. f. Physiol. Chem.*, 1938, **252**, 137.
‡ Haarmann and Schroeder. *B.Z.*, 1938, **296**, 35.

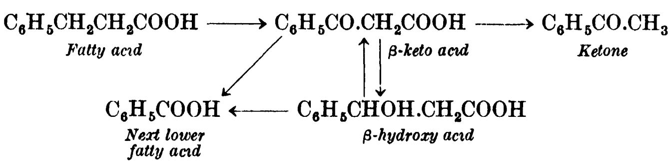
THE OXIDATION OF FATTY ACIDS

Any theory of fatty acid oxidation must account for the fact that fatty acids with any number of carbon atoms up to 20, both saturated and unsaturated, have to be oxidised. Secondly, it should be remembered that all the natural fatty acids contain an even number of carbon atoms.

(*a*) *β-oxidation.* The normal animal combusts fat to carbon dioxide and water and generally, therefore, no oxidation products are excreted in the urine. Evidence as to the nature of the intermediate steps has been obtained, however, by administering to animals the phenyl derivatives of fatty acids and examining the urine for the end products. The advantage of the introduction of the benzene ring into the fatty acid molecule is that the tissues cannot readily disrupt the benzene ring, which must therefore be excreted, and which may carry with it a partially oxidised residue of the fatty acid molecule to which it was attached. Use has been made of such methods by Knoop and Dakin. Knoop (1904) * found that when a series of phenyl fatty acids, beginning with phenyl acetic acid, were given to animals, one of two products only appeared in the urine, either phenyl formic acid (benzoic acid), or phenyl acetic acid, in accordance with the following scheme :—

Acid administered		No of C atoms in fatty acid chain	Product excreted	No of C atoms lost
Phenyl formic acid (benzoic acid)	C_6H_5COOH	1	C_6H_5COOH	0
,, acetic acid	$C_6H_5CH_2COOH$	2	$C_6H_5CH_2COOH$	0
,, propionic acid	$C_6H_5(CH_2)_2COOH$	3	C_6H_5COOH	2
,, butyric acid	$C_6H_5(CH_2)_3COOH$	4	$C_6H_5CH_2COOH$	2
,, valeric acid	$C_6H_5(CH_2)_4COOH$	5	C_6H_5COOH	4

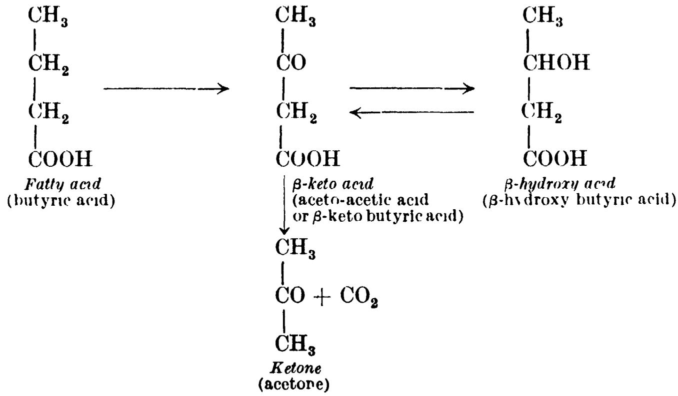
Since the number of carbon atoms lost was always an even number, this suggested that the fatty acids lost their carbon atoms two at a time, the odd-numbered acids giving rise to benzoic acid and the even-numbered to phenyl acetic acid. This work was extended by Dakin,† who showed that further intermediate products could be isolated from the urine ; thus when phenyl propionic acid was given, benzoyl acetic, mandelic, cinnamic and benzoic acids were obtained in accordance with the following scheme :



These two lines of evidence are the basis on which the so-called theory of β-oxidation of fatty acids has been evolved. When a fatty acid is oxidised, the first product is a β keto acid, which may undergo a number of further changes ; it may be oxidised at the carbonyl group to the fatty acid with two carbon atoms less ; it may lose carbon dioxide to give the corresponding ketone, or it may be reduced to give the corresponding

* Knoop. *Beit. Z. Chem. Physiol. Path.*, 1905, **6**, 150.
† Dakin. *J. Biol. Chem.*, 1908, **4**, 419 ; **5**, 173, 303.

β-hydroxy acid. Applied to butyric acid, this series of products is as follows :



Thus, in the normal oxidation of a saturated acid like stearic acid, $C_{18}H_{36}O_2$, after the corresponding intermediate products had been first produced, palmitic acid, $C_{16}H_{32}O_2$, would be formed by loss of two carbon atoms. This process of losing two carbon atoms at a time is repeated with the production next of myristic acid, $C_{14}H_{28}O_2$, and similarly through successive stages to butyric acid, $C_4H_8O_2$, and finally to carbon dioxide and water. Two things must be pointed out. Firstly, that the higher intermediate products have not been isolated, probably because of technical difficulties, and because the further oxidation is rapidly and completely achieved, so that these products never accumulate. Secondly, that acetone arises from aceto-acetic acid, which in turn comes from butyric acid, and that acetone cannot therefore arise from odd-numbered fatty acids.

There is much evidence in support of the view that β-oxidation is one of the normal mechanisms by which fatty acids are oxidised. Thus in any state in which carbohydrate cannot be adequately oxidised (diabetes mellitus), or in which there is insufficiency of carbohydrate for oxidation (starvation), excess of fat has to be burnt in order to supply sufficient energy. In this condition a state of *ketosis* occurs in which there is excretion in the urine of aceto-acetic and β-hydroxy butyric acids and acetone. These are precisely the products which, as we have seen, should be obtained on the β-oxidation theory, if, as in these conditions, fatty acid oxidation were incomplete.

The oxidations in question appear to be carried out by the liver, and liver perfusion investigations bear this out. If odd- or even-numbered fatty acids are added to defibrinated blood used for perfusing a liver, there is an increased amount of acetone produced when even-numbered fatty acids are used, but not in the case of odd-numbered ones.*

Further confirmatory evidence has come from studies by the Warburg technique of the oxidation of fatty acids added to liver slices. When lower fatty acids were added to liver slices, only those which possessed an even number of carbon atoms gave rise to additional aceto-acetic acid and, further, in the liver, aceto-acetic acid was the final oxidation product.† Thus the evidence that fatty acids may be oxidised by a process of β-oxidation seems strong, although some evidence points to the fact that other

* EMBDEN, SALOMON and SCHMIDT. *Beit. Z. Chem. Physiol. Path.*, 1906, **8**, 129 ; EMBDEN and MARX, *ibid.*, 1908, **11**, 318.

† QUASTEL and WHEATLEY. *Biochem. J.*, 1933, **27**, 1753.

methods of oxidation of fatty acids are also available, and these will now be mentioned.

(*b*) *γ- and δ-oxidation.* It very often happens that the products which are obtained when substances are oxidised in the laboratory with hydrogen peroxide are those which are formed in the body during oxidation of those same substances; in fact hydrogen peroxide is the oxidising agent most resembling biological oxidations in its action. It is therefore of peculiar interest that Raper * found, by oxidising the ammonium salts of fatty acids with hydrogen peroxide, that γ- and δ-keto acids were also produced, thus showing that in his experiments γ- and δ-oxidation occurred. Further oxidation of these particular products gave rise to succinic acid. If these findings could be substantiated in the animal body, it would be of considerable importance, for succinic acid is readily convertible to glucose in the body and thus a means whereby sugar could arise from fatty acid would be apparent.

Oxidation of a substance entails the addition of oxygen or the loss of hydrogen from the substance and clearly the loss of hydrogen atoms from a fatty acid with the production of unsaturated acids would be an oxidation process. This would thus be a desaturation process, just as succinic acid by the loss of two hydrogen atoms is readily converted to fumaric acid in the liver and elsewhere. This question of desaturation will be discussed later under the section on the function of the liver in fat metabolism.

(*c*) ω-oxidation. A further form of fatty acid oxidation has come to light.† It was observed that on administration of the triglycerides of some fatty acids, dicarboxylic acids with the same number of carbon atoms could be isolated from the urine, *i.e.* the terminal methyl group of the fatty acid had been oxidised to a carboxyl group. The biggest yield of such products was obtained with the triglyceride of capric acid, $CH_3(CH_2)_8COOH$, which gave rise in the urine to sebacic acid, $HOOC(CH_2)_8COOH$, with smaller quantities of the corresponding dicarboxylic acids with six and eight carbon atoms, *i.e.* suberic acid, $HOOC(CH_2)_6COOH$, and adipic acid, $HOOC(CH_2)_4COOH$. The corresponding dicarboxylic acid may then undergo β-oxidation with the production of its lower homologues with two, or multiples of two, carbon atoms less.

It will be clear from what has been said that fatty acids may be oxidised in the body by a number of different paths; further work is required to determine to what extent and under what conditions these different paths are followed; present knowledge suggests that the β-oxidation process plays the predominant part.

(*d*) *The Anti-ketogenic Action of Carbohydrate.* It has already been mentioned that when fat is being utilised, with relative suppression of carbohydrate oxidation, the so-called "ketone bodies" appear in the urine. This does not occur when adequate carbohydrate or other glucose-yielding (antiketogenic) substance is being burnt at the same time. It has been suggested that this is to be interpreted as meaning that normally, in the later stages of fat oxidation, compounds are formed with carbohydrate and that it is these carbohydrate-fat complexes which are finally oxidised. Ketosis would thus occur because these compounds are not formed when carbohydrate oxidation is suppressed, and this is the basis of the expression that fat is burnt in a "fire of carbohydrate." No rigid proof is yet forthcoming, however, that such compounds are formed. It might appear more reasonable to believe that the normal animal is not fitted to derive its energy almost exclusively from fat and, if forced to do so, the mechanism is found partially wanting in that the penultimate products of fatty acid oxidation are not themselves completely oxidised. It is nevertheless curious that, having oxidised the long chain fatty acids to simple products containing only four carbon atoms, these products should then in part be excreted. A possible explanation is that, since these products are being produced in excessive amounts and since they are water-soluble, they are passed out into the urine, whereas normally the small amount being produced at any

* Clutterbuck and Raper *Biochem. J.*, 1925, **19**, 385; 1926, **20**, 59.

† Verkade and Lee. *Biochem. J.*, 1934, **28**, 31; *Z. physiol. Chem.*, 1934, **225**, 230; **227**, 213.

given time is almost completely oxidised (*cf.* lactic acid production in severe muscular work). The excretion of aceto-acetic acid is in any case a protective mechanism, since that substance is toxic.

This anti-ketogenic action of carbohydrate, as it is called, has been investigated by dietary studies in which mild acetonuria has been induced by a restricted diet. Such studies * show that when the molecular ratio of ketogenic to anti-ketogenic substances in the food exceeds two, acetone bodies appear in the urine. The anti-ketogenic substances will, of course, include not only dietary carbohydrate, but sugar which may be derived from glycerol, or from the straight-chain amino acids such as alanine, whilst the ketogenic fraction will include, not only the fatty acids, but also those amino-acids, such as tyrosine, which give rise in their metabolism to aceto-acetic acid. These results are clearly of importance from the point of view of diabetic feeding and simply expressed they mean that fat oxidation begins to be incomplete when the amount of "fat" in the diet is more than twice that of the carbohydrate actual and potential.

Ketosis is produced or augmented by the injections of certain extracts of the anterior pituitary body—the so-called ketogenic fraction. Conversely, extirpation of the anterior pituitary tends to reduce or abolish the state of ketosis produced in various ways, as *e.g.* by removal of the pancreas. Similar claims regarding the suprarenal cortex have also been made, and there are grounds for a growing belief that the effects of pituitary extirpation are really due to a control exerted by that organ upon the size and activity of the suprarenal cortex.

THE LIVER AND FAT METABOLISM

Many pathological and experimental findings indicate that the liver plays a special part in fat metabolism. The most striking fact in this connection is that fat is transported to the liver in many conditions. Normally the liver contains some 3 per cent. of lecithin and 1 per cent. of glyceride. In the variety of conditions which may be classed together as fatty livers, large amounts of glyceride may appear, increases up to 70 times the normal amount having been observed. These fatty livers appear in the early stages of starvation, in poisoning by phlorrhizin, chloroform, alcohol, phosphorus and a variety of other chemical agents; they occur after pancreatectomy, in clinical and experimental diabetes, in pernicious anæmia and a number of other pathological states. They may be induced also by diets high in fat or containing cholesterol. Although transportation of fat to such tissues as the heart and kidney also occurs, its frequency is very much less, as is also the actual degree of fat infiltration.

Investigations to determine the source of this fat, by experiments in which animals have been starved to remove the depôt fat, then fattened on a "labelled" fat, *e.g.* on fat containing deuterium instead of some of its hydrogen atoms,† and finally poisoned with carbon tetrachloride, or starved, to cause deposition of fat in the liver, indicate that the mobilised liver fat has come from the depôts. But if "labelled" fat containing deuterium is given until the depôts contain that fat, and the animal is then given a diet low in protein but rich in carbohydrate, the liver fat contains no deuterium and so has probably been formed from carbohydrate. Hence it appears that new-formed fat appears directly in the liver, but mobilised fat is transported thither, so that the problem resolves itself into a consideration of why depôt

* Schaffer. *J. Biol. Chem.*, 1921, **47**, 433, 449; 1921, **49**, 143; 1922, **54**, 399.
† Barrett, Best and Ridout. *J. Physiol.*, 1938, **93**, 367.

fat appears in the liver. It is to be recalled that the fatty acids present in the liver fats have a higher iodine value than have those of depôt fat, but one not much more than half that of the acids present in the liver as phosphatide. This and other findings led Leathes * to suggest that depôt fatty acids are mobilised and transferred to the liver, where they undergo a process of desaturation and conversion to phosphatides. They are then carried to the tissues for oxidation in this now more unstable form or else are, at least partially, oxidised in the liver itself. The proofs that ketone bodies are formed in the liver, including the fact that after removal of the liver the ketosis following pancreatectomy or injection of the pituitary ketogenic fraction is reduced or abolished, supports the view that the greater part of the oxidation of fats takes place in the liver. There is a close relationship between the rate at which fat is transported to the liver and the extent of ketosis. Thus, adrenalectomy reduces both ketosis and the fat of the liver induced by injection of anterior pituitary extracts. If we accept the view that the oxidation of fats proceeds in the liver up to the stage at which 4-carbon ketone bodies are produced, then it would seem probable that these substances, set free into the blood stream, are finally oxidised, together with glucose—also released by the liver—in the tissues.† That certain tissues, *e.g.* heart muscle, can utilise β-hydroxybutyric and has been demonstrated beyond question.‡

The hypothesis of desaturation in the liver was based on two lines of work. The first of these was that when highly unsaturated oils are administered to animals, the liver acids become even more unsaturated than those of the oils in the diet, which was interpreted as evidence that desaturation had occurred.§ The second was that of Hartley,|| on the nature of the fatty acids of pig liver and pig depôt fat. He found in pig liver an oleic acid having the double bond between the twelfth and thirteenth carbon atoms ($\Delta^{12\cdot 13}$), whilst ordinary oleic acid ($\Delta^{9\ 10}$) was absent. In the lard, however, ordinary oleic acid only was present. Further, in the liver there was linoleic acid with two double bonds ($\Delta^{9\ 10\ 12\ 13}$), which, however, was absent from depôt fat. These findings suggested that the mobilised depôt fat had been transferred to the liver, which possessed the power of introducing a double bond between the twelfth and thirteenth carbon atoms, *i.e.*

Depôt fatty acid		*Liver fatty acid*
oleic acid $\Delta^{9\ 10}$	$\longrightarrow$	linoleic acid $\Delta^{9\ 10\ 12\ 13}$
stearic acid	$\longrightarrow$	oleic acid $\Delta^{12\ 13}$

Although these two lines of work appeared to provide strong evidence in favour of desaturation, more recent work seems to throw considerable doubt on them. In the first place, the original results of Leathes could be accounted for by the preferential uptake by the liver of the more unsaturated acids of the fats in the diet, and, as will appear later, this has been shown to occur.¶ Further, investigations by other workers ** have failed to demonstrate the particular liver acids which were originally found by Hartley, and which appear therefore not to be of general occurrence. Thus the original evidence in favour of desaturation of fatty acids in this particular manner in the liver has lost much of its value, and it is further weakened by the recent claim that, as explained in an earlier chapter, certain fatty acids are essential dietary constituents in just the same way as certain amino-acids are essential.†† One of these fatty acids is

* Leathes and Raper. "The Fats," London, 1925

† Blixenkrone-Møller. *Z f physiol. chem.*, 1938, **252**, 117.

‡ Waters *et al.* *Amer. J. Physiol.*, 1938, **122**, 542.

§ Leathes and Meyer-Wedell. *J. Physiol.*, 1909, **38**, Proc. xxxviii.

|| *J. Physiol.*, 1909, **38**, 353.

¶ Sinclair. *J. Biol. Chem.*, 1929, **82**, 117.

** Turner. *Biochem. J.*, 1930, **24**, 1327; Channon, Irving and Smith. *Biochem. J.* 1934, **28**, 852.

†† Burr and Burr. *J. Biol. Chem.*, 1929, **82** 345; 1930, **86**, 587; Burr, Burr and Miller. *J. Biol. Chem.*, 1932, **97**, 1.

linoleic acid, which on the desaturation hypothesis might be expected to be readily produced in the liver by the desaturation of either depôt stearic or oleic acids.

Although the evidence in favour of this particular type of desaturation has lost much of its force, this must not be regarded as disposing of desaturation as a biological process, and recent work seems to suggest that desaturation does in fact occur.* Incubation and other experiments with livers, both normal and fatty, suggest that the liver can desaturate fatty acids and it is tentatively suggested that this desaturation, which may be carried out by other tissues, also occurs in the $\alpha-\beta$ positions. We must therefore conclude that desaturation probably occurs, although the original evidence and the particular type of desaturation which was originally put forward is largely disproved.

The common C_{18} acids in Nature are oleic, linoleic and linolenic acids. These products, presumably built up in the plant from carbohydrate, have their double bonds in the 9 : 10; 9 : 10. 12 : 13 ; 9 : 10. 12 : 13. 15 : 16; positions respectively. The occurrence of these double bonds symmetrically disposed, three carbon atoms from each other, might suggest that they have been evolved by a synthesis from a three-carbon unit, which has been produced from sugar by some such means as has been indicated in an earlier paragraph. Animals on a fat-free diet build up much oleic acid with traces of higher unsaturated acids (arachidonic acid), although the ability to synthesise linoleic acid is absent, while linolenic acid does not occur. It remains for future research to show why oleic acid with one double bond can be apparently synthesised with ease, whereas linoleic acid with two double bonds cannot.

Lipotropic Action. Investigation of the fatty liver problem has received considerable impetus by the discovery of Best and his colleagues of the control of liver fat—the so-called *lipotropic action*, exerted by choline.† On diets which are free from choline, large amounts of fat appear in the liver, whereas if choline be present in the diet there is no accumulation of liver fat. Furthermore, in experiments in which fatty livers have been produced it is possible to cause the disappearance of the fat by the administration of choline, whereas in the absence of choline the liver fat remains at its abnormally high level. This action of choline in liver fat control is clearly of great importance, because it enables us by dietary means to produce fatty livers which are very much more physiological than those hitherto available; furthermore, it allows the amount of liver fat to be controlled at will by the inclusion of suitable amounts of choline in the diet; it has also provided a new method whereby the *rôle* of the liver in fat metabolism may be further investigated. The amounts of choline which are effective may be quite small, for in the rat, 5 milligrammes per day has been found to prevent a rise of some 12 per cent. of fat, expressed as the wet liver weight.

The mechanism of this choline action is as yet unknown, although an obvious possibility is that it provides the base choline for phosphatide synthesis, and in this case might link up with the hypothesis of lecithin as a carrier of fatty acids. It appears from present work then, that choline is an essential dietary constituent.

The effect of choline in preventing the fatty liver was discovered through the observation that depancreatised dogs developed fatty livers. Extracts of pancreas contain a lipotropic factor,‡ but it is disputed whether this may be attributed only to the presence of choline in such extracts.

* For references see ARTOM, " Annual Review of Biochemistry," 1934–35, 212.
† BEST. *Lancet*, 1934, 1274 ; BEST and HUNTSMAN. *J Physiol.*, **83**, 255
‡ CHANNON *et al.* *Bioch J.*, 1938, **32**, 1332.

Proteins also exert a lipotropic action, due perhaps to certain constituent amino cids.*

FATTY DEGENERATION. In certain pathological conditions various organs of the body, such as the liver, undergo what has been described as *fatty degeneration* and this has long been interpreted as a conversion of the proteins of the cells into fat. We have seen already that such a conversion does not occur in the normal animal, and so-called fatty degeneration is due to a mixture of two contributing factors. The first of these is *fatty infiltration, i.e.* mobilisation from the depôts occurs, as has already been discussed. Secondly, the protoplasm of cells contains "fat" in such a condition that its presence cannot be detected by histological means, probably because it is in some form of adsorption complex with the proteins. Hence the presence or absence of visible fatty globules affords very little clue to the quantities of fat in the cells. In various diseases and toxic conditions this fat is unmasked, due to the effect of various agents in breaking down the intimate cell structure and this is the second factor involved in fatty degeneration. The expression "*unmasked fat*" used in histology and pathology refers to such protoplasmic fat.

THE RÔLE OF PHOSPHOLIPIDES IN THE TISSUES

On the grounds of its chemical structure (70 per cent. fatty acid) and of the peculiar physical property of forming almost true solutions in water, lecithin obviously suggests itself as one of the means whereby fatty acids could be transported in the body. This brings us to consider whether phospholipides are integral parts of cell structure, metabolically inert from the point of view of fatty acid metabolism, or whether they are actively concerned in the latter process, or both.

There is considerable evidence that formation of phospholipides may occur in the intestine, from fatty acids; when labelled fatty acids were given they were traceable in the phospholipides of the intestinal mucosa after a short interval,† and it has been suggested that fatty acids may thus be transported in part into the portal blood and not, like neutral fat, into the lymph.

The total ether-soluble material present in such tissues as the normal liver, heart, kidney, &c., consists largely, not of glycerides, but of a mixture of phosphatides. If these two groups of constituents be separated from one another and the fatty acids prepared from them by hydrolysis, the phosphatide acids are always found to be more unsaturated than the glyceride acids, and these in turn are usually more unsaturated than those of the depôt fat. Some illustrative figures are as follows:

	Iodine values of:		
	Total fatty acids.	Fatty acids of phosphatide.	Fatty acids of glyceride.
Liver	113	134	76
Heart	107	128	67
Kidney	92	117	84
Spleen	90	98	75
Lung	66	77	60

The total amount of ether-soluble material in such tissues as those given in the table varies between some 2 and 4 per cent. and, depending on dietary

* CHANNON *et al. Bioch. J.*, 1938, **32**, 976.
† SINCLAIR. *J. Biol. Chem.*, 1936, **115**, 211.

conditions to some extent, one-half to two-thirds or more may consist of phosphatide with the remainder as glyceride, cholesterol and cholesteryl esters.

The problem then is to decide why phospholipides occur in all tissues and why they are more unsaturated than are the glycerides. Much work has been done to attempt to determine whether lecithin is actively involved in fat metabolism as such. As we have seen when dealing with absorption, there is *primâ facie* evidence that formation of lecithin in the intestine, at any rate, may play an active part either in the absorption of fat or in the processes immediately following it. Further, in certain conditions, the amount of liver phosphatide appears to be changed after a fatty meal, and its fatty acids modified by the fat of the diet; thus iodised fats appear both in the liver and blood corpuscle phosphatides after administration.* Further, the effect of choline † in controlling the level of liver fat also suggests an active *rôle* for lecithin. These points all, superficially at least, lead to the same conclusion.

On the other hand, the phosphatide of most tissues shows very little change in extreme dietary variations, and such tissue as brain, which contains much phosphatide, apparently does not utilise fats. Moreover, it is particularly the unsaturated fatty acids which are built up into phosphatides in the intestine, and which are retained with tenacity in the tissues. We may conclude then by saying that, although the chemical and physical properties of lecithin make it suitable from the point of view of fat transport and oxidation, the clear evidence so far adduced in favour of its active participation is relatively slight, save perhaps in the case of the liver and intestinal mucosa.

This brings us then to the consideration of the structural functions of phospholipides. These are essential cell constituents, forming an integral part of the cell membrane, and it might be anticipated, therefore, that even though these substances contain 70 per cent. of a fatty acid in their molecule and therefore have a high calorific value, they could not be called upon as reserve material without the destruction of the cells in which they are present. That this is so is indicated by much work carried out by Maeyer and Terroine.‡ The French School of workers make use of the expression the "*constant element*," by which they mean the fatty acids present in one form or another which cannot be called upon in time of need. That this constant element exists has been shown by studying the lipoids of animals which have died from inanition; the larger part of the fatty acids of these are present as phosphatides and are constant both in amount and composition. This would imply that the phosphatide fatty acids of a given tissue are characteristic of that tissue and cannot be varied by diet. Whilst the constancy of amount seems to have been confirmed by further work, it has been shown that the nature of the fatty acids, particularly of intestinal mucosa and liver, may be substantially modified by diet. It is, however, interesting to note that the degree of unsaturation of phosphatide fatty acids does seem to be more or less characteristic of the tissue in that the iodine value of the acids in the different tissues seems to run in the same order.

As to the part taken by this essential fat of the working cells, in the life of the cell, our knowledge is still very scanty. Fatty acids enter into the constitution of the phospholipides and glycolipides which form important

* ARTOM. *Arch. inter. Physiol.*, 1933, **36**, 101; ARTOM and PERETTI. *Arch. inter. Physiol.*, 1933, **36**, 351.

† BEST and HUNTSMAN. *J. Physiol.*, 1935, **83**, 255.

‡ TERROINE. "Physiologie des substances grasses." Paris, 1919.

constituents of the protoplasm and limiting membrane of every cell. As constituents of the membrane itself, fatty substances therefore have a protective action and also regulate the passage of substances into the cell across the membrane. The presence of the phospholipides as integral constituents of all protoplasm suggests that they may play a part in the normal transformations which occur within the cell, and may represent, so to speak, the currency into which fat is transformed in order to participate in the vital processes, and to be transported and utilised for the needs of the cell. Much work also indicates that there is a physiological antagonism between cholesterol and lecithin, which presumably is related to their physical properties.

CHANGES DURING ABSORPTION

As we have seen earlier, the fatty acids are absorbed in the small intestine and are resynthesised in their passage through the intestinal epithelium. some to neutral fat and some to phospholipide. The fat resulting from this resynthesis may, for several reasons, be of a different character from that of the food. In the first place, the ease of absorption of fatty acids varies with their physical properties. Unsaturated liquid acids are very readily absorbed, especially to form phospholipide, whilst saturated acids are less well absorbed with increasing length of the carbon chain, so that the higher saturated acids are poorly absorbed unless unsaturated acids are being absorbed at the same time. Hence, in some conditions, higher fatty acids may not be absorbed, but passed into the fæces. Further, bile and other secretions contain certain amounts of fatty acids and these too may be absorbed with the food fat and cause some modification in its condition.

The original food fatty acids appear in the chyle, and eventually in the blood, as a mixture of phosphatide, glyceride and cholesteryl esters, glyceride usually being in a vastly greater proportion.

This brings us to consider whether the various lipides of the blood show any changes after a meal. Here the evidence is not conclusive. There is, of course, an increase in the neutral fat in the blood, and lipæmia may develop. With this there is also an increase in phosphatide and cholesteryl ester at different times after absorption, and the extent of these changes is different in corpuscles and plasma. Attempts have been made to interpret the findings as meaning that the phosphatide or cholesteryl ester play an active part in the transport of fatty acids *; another possible view is that with the arrival of food fatty acids into the blood, considerations of physico-chemical equilibrium may require readjustment of the proportions of the phosphatide and cholesteryl ester present. Since cholesterol has a melting point of 150° C., while its unsaturated esters are liquid at body temperature, it may be that these unsaturated esters occur on this account; but at this stage the somewhat conflicting evidence would make conclusions premature.

Post-absorptive blood fat in the case of some animals consists of phosphatide and cholesteryl esters, with very little glyceride, and in this case it is found that the fatty acids of the cholesteryl esters have a higher iodine value than do those of the phosphatide.† The significance of this has yet to be established.

STEROL METABOLISM

The metabolic fate of cholesterol and the other sterols is poorly understood at the present time. Cholesterol can be synthesised in the body ‡ and is a constituent of all cells. It is absorbed from the intestine, provided there is sufficient fat in the diet, and the absorption depends also, of course, on the presence of bile. Plant and some other sterols are poorly absorbed from the alimentary tract, if at all. Bile always contains cholesterol, and the excretion of sterol in the fæces is largely in the form of

* Bloor. *J. Biol. Chem.*, 1915, **23**, 317; 1916, **24**, 447.

† Bloor. *J. Biol. Chem.*, 1924, **59**, 543; Channon and Collinson. *Biochem. J.*, 1929, **23**, 1212.

‡ Channon. *Biochem. J.*, 1925, **19**, 424.

coprosterol. Just as fatty acids are converted to neutral fat during absorption, so cholesterol is also in part esterified with fatty acids. The ability of the animal to destroy cholesterol appears to be limited, because if 1 per cent. of cholesterol is present in the diet of rats or rabbits, large quantities are deposited in the different organs, mainly in ester form, and intense fatty livers in which enormous quantities of glyceride also occur may be produced by such means. Further investigation of this problem may have clinical significance. Deposition of cholesterol in the tissues seems to be general, although brain is an exception. In such experiments as these part of the cholesterol is, however, oxidised to unknown compounds. Various bile acids which are being continuously formed are almost certainly derived from cholesterol, as are the sex hormones.

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4. CARBOHYDRATE METABOLISM

The Blood Sugar

The carbohydrates present in the body consist of the sugar in the blood and tissue fluids, and the glycogen stored in the tissues, principally in the liver and muscles. All the carbohydrates in an ordinary diet are ultimately transformed in the alimentary tract into one or more of the three monosaccharides, glucose, fructose and galactose. These three, together with mannose, are utilisable from the blood by the higher animals. As we have seen in an earlier chapter, they are convertible, one into the other, and it is believed that conversion into glucose does take place in the body. Glucose is certainly present in the blood,* but whether it is the only simple carbohydrate there is not definitely settled; no others have so far been isolated.

It is usual in quantitative determinations to express blood sugar as milligrams of glucose per 100 c.c. of blood. Accurate determination of sugar in the blood is difficult. Some of the methods in common use give the total reducing power, but this is not all due to glucose; human blood contains reducing substances other than sugars which may be equivalent to no less than 27 mg./100 c.c. of glucose,† and it is similar with other species. Some methods include more of these substances along with the glucose than others,‡ but when the same method is used the error due to "non-sugar" substances is constant for a given species, and when it is deducted from the total "apparent glucose," the "true glucose" is obtained. The non-sugar reducing substances (mainly glutathione and thioneine) are principally contained in the corpuscles, 100 c.c. of which contain 47 mg. (expressed as glucose), while plasma only contains 10 mg. per cent.§ In consequence of this high concentration in the corpuscles of reducing substances other than sugar, the corpuscles in normal blood have often been stated to have a glucose concentration 1·1 times that of serum, whereas in fact the ratio is only 0·77. Fortunately for most of the investigations on this subject, since the non-sugar substances are practically constant, variations in the apparent blood-sugar as usually determined do usually represent variations in the true glucose content.

The glucose of the blood plasma is apparently in equilibrium with that in the tissue fluids. Although the body only has, say, 2 l. of blood plasma, which would contain about 4 g. of glucose, the tissue fluid amounts to about 20 l., and so represents a considerable store of glucose—at least 20 g. Consequently if glucose is suddenly added to the circulating blood, as by intravenous injection, the blood sugar is not raised so much as might be expected,

* WINTER. *Biochem. J.*, 1930, **24**, 851.
† SOMOGYI. *J. Biol. Chem.* 1927, **75**, 33.
‡ HERBERT and BOURNE. *B.M.J.*, 1931, i., 94
§ SOMOGYI. *J. Biol. Chem.*, 1928, **78**, 117.

since, after a few minutes, glucose has diffused out into the tissue fluid until equlibirium is reached. If, for instance, 50 g. of glucose were given intravenously, the blood sugar would not rise to 1·3 per cent., but only to about 0·35 per cent. Conversely, the withdrawal by the tissues of 2 g of glucose would not cause the blood sugar to disappear, but would scarcely lower it at all.

During the absorption of a large carbohydrate meal, considerable quantities of monosaccharides enter the blood, and the amount of sugar in the blood is increased. When the absorption is rapid, as, for example, after giving 50 g. of glucose to a fasting person, the systemic blood sugar is, in fact, raised for a while to about double the fasting level; the portal blood sugar is higher still, and runs closely parallel with the systemic blood sugar. This procedure

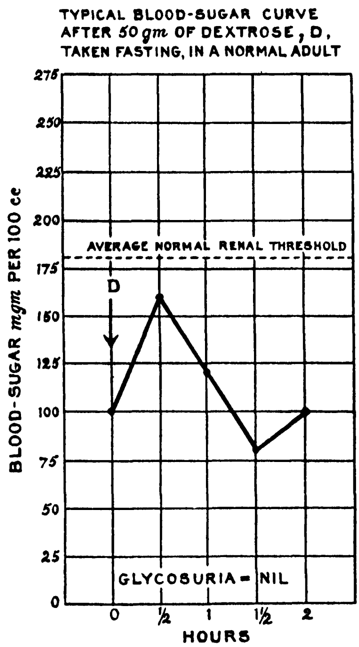

FIG. 569. (HARRISON.)

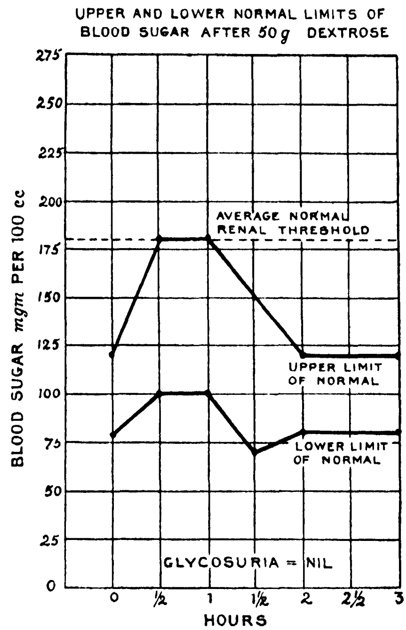

FIG. 570. (HARRISON.)

of giving glucose and estimating the blood sugar at half-hourly intervals for the next two or three hours is called the *glucose tolerance test.**

For this purpose the fasting level of the blood sugar is determined; it is usually from 80 to 120 mg./100 c.c. (*i.e.* 53 to 93 mg./100 c.c. true sugar). Then 50 g. of glucose dissolved in water is taken by the mouth. Samples of blood are drawn by puncture, each half hour for three hours, and their sugar content determined. Within thirty minutes after taking the glucose the blood sugar will be found to have risen to a level not usually exceeding 180 mg./100 c.c. (Figs. 569, 570); in one hour the amount has begun to fall, and at the end of one and a half to two hours the blood sugar has returned to normal again, or it may be even a little below (Fig. 569). The blood sugar curves so obtained do not vary greatly in normal persons, and are of considerable value clinically in investigating disorders of carbohydrate metabolism, such as diabetes. It is usual to test the urine for

* *v.* HARRISON. "Chemical Methods in Clinical Medicine." (Churchill.) 1930, p. 101.

lucose at intervals after taking the glucose; none should be present. If n ordinary meal containing insoluble carbohydrates capable of digestion, ıch as starch, be taken instead of glucose, the rise is of slightly longer uration, owing to the relatively slow formation and absorption of the roducts of digestion (Fig. 571).

It is important to note that although such alterations of the blood sugar ccur, these are normally never very great. In most warm-blooded animals ıe blood sugar is rarely below 80 mg./100 c.c., and does not normally rise bove 200 mg./100 c.c. This is because there is a well-controlled means for ægulation of the blood sugar concentration, *i.e.* of the sugar available to the ssues. Even during prolonged tarvation glucose does not disppear from the blood, nor even ıll notably below the amount resent after a fast of only a few ours. Conversely, even after the eaviest carbohydrate meal, the ystemic blood sugar in normal ubjects does not rise above about 80 mg./100 c.c.

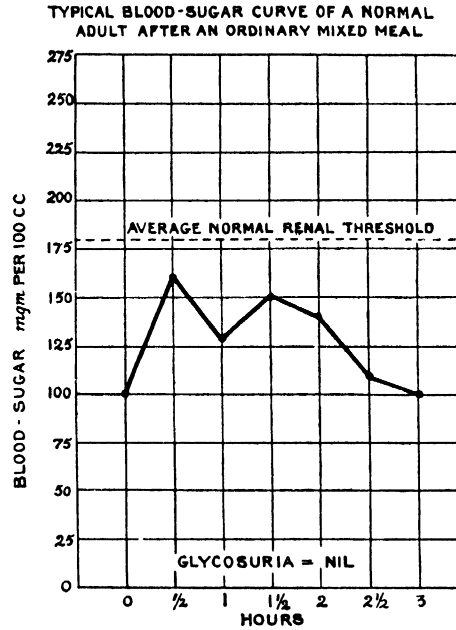

Fig. 571. (Harrison.)

This relatively steady level f the blood sugar depends on an djustment of the rates at which ugar enters and leaves the blood tream. If the blood sugar reıains constant, it is because, like he water level in a tank, the ates of inflow and outflow are qual. If one is greater than the ther, the level must rise or fall *ontinually* until they again beome equal. Under normal condiions the regulating mechanism s 'set' to a glucose level of about 0 mg./100 c.c., but under abnorıal conditions it may be set at higher or lower levels; when this is the case ve cannot infer that the rates of entry or exit of glucose have undergone any rolonged change—all we can say is that the regulation is set at a higher or ɔwer level.

The following scheme summarises briefly the source of gain and loss of ugar to the blood :—

Gain.	*Loss*
From absorbed sugar.	Removed by excretion.
From liver glycogen.	Removed by diffusion into tissue fluid.
Added by diffusion from tissue fluid.	Destroyed in blood.
	Removed by liver and other tissues.

One method by which glucose may be removed from the blood and issues is by *glycolysis*. This is due to the action of an enzyme by which he glucose is converted into lactic acid. The enzyme is present in the eucocytes, and also in various tissues. Glycolysis occurs in the circulating

blood, particularly in its transit through the lungs,* and accounts for th constant presence of lactic acid in the blood. The glycolysis in she blood may be prevented by the addition of small amounts of sodium fluoride. Sugar is constantly being removed from the circulating blood for use b the cells of the tissues. Hence we find that the venous blood from mos parts contains less glucose than is present in the arterial blood, the differenc being a few mg./100 c.c. Since the sugar concentration of the arterial blood approximately constant, it follows that, even in starvation, glucose must b regularly added to the blood at about the same rate as that at which it i removed. When glucose is entering the blood from the alimentary cana much of it, as we shall presently see, is rapidly removed from the blooc some of it being burned and some apparently deposited in the form c glycogen in the muscles and other tissues. Nevertheless, it is not usuall removed quite as rapidly as it enters, so that the systemic blood sugar doe show a slight rise ; this tends to bring about a still faster rate of removal, s that by the time the blood sugar level has reached 180 mg. p.c. depositio and combustion keep pace with absorption. In this way many norma persons can dispose of 200 or 300 g. of carbohydrate at a meal. Should th rate of removal be unable, however, to keep pace with the rate of absorptio into the blood, or, should glucose be *rapidly injected* into the blood stream then the excess of glucose is removed from the blood stream in another way namely, by excretion by the kidneys, and appears as glucose in the urine, a condition called *glycosuria*. The presence of an excess of sugar in the bloo is called *hyperglycæmia*, but when sugar is excreted in the urine its concentra tion therein is always much greater than that in the blood ; if the sugar i the blood amounts to, say, 400 mg./100 c.c., the urine may contain 5 or per cent. of glucose.

At the other extreme, lowering of the blood sugar beyond a certain leve cannot be effected by complete deprivation of food, however prolonged This means that the starving animal is able to produce blood sugar fron some source within the body. The most readily available source is th glycogen store, especially that in the liver, and it is this which is first calle upon in the early stages of starvation. Yet, even after prolonged starvation the liver is found to be not quite free from glycogen, though the amoun present in it may be 0·20 per cent. or less ; the muscle glycogen is als reduced in starvation, but likewise never disappears. Glycogen must therefore, under such conditions be manufactured continuously within th body, probably in the liver, from some non-carbohydrate source, a proces known as *glyconeogenesis*.

If for any exceptional reason the sugar content of the blood can be brough much below 70 mg. p.c., severe symptoms result, and ultimately, if th fall continues, death occurs. This condition of *hypoglycæmia* may be brough about by the action of insulin, a hormone derived from the islets of th pancreas. In the rabbit an injection of insulin sufficient to cause reductio of the blood sugar to about 45 mg. p.c. causes severe convulsive seizures followed by coma, fall of temperature and death from respiratory failure In man, subjective symptoms are induced when the blood sugar falls to 75 mg. p.c. These symptoms are extreme hunger, a sense of fatigu and anxiety, vasomotor phenomena, such as pallor or flushing, and genera sweating. As the blood sugar falls still further, mental disturbances delirium and finally profound coma supervene. These serious consequence show that a certain minimal quantity of glucose must always be present i

* EVANS, HSU & KOSAKA. *J. Physiol*, 1934, **82**, 41

† LOVATT EVANS. *J. Physiol*. 1922, **56**, 146.

the blood and tissues for the maintenance of the normal functions of the central nervous system.

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LIVER GLYCOGEN FORMATION. It was discovered by Claude Bernard * that the blood of the hepatic veins nearly always, but particularly in fasting animals, contains more sugar than is present in the blood taken simultaneously from the portal vein, *i.e.* the liver adds sugar to the blood which passes through it. That the liver can produce glucose he showed by a simple experiment; the liver removed from an animal and kept warm for some time was found, on extraction with water, to contain considerable amounts of glucose. He next performed the classical experiment with the 'washed liver' †; a liver was removed from an animal immediately after death and its vessels washed out with ice-cold water or salt solution; the tissue was then cut up and thrown into boiling water, in which it was thoroughly ground up. The extract, after separation of the coagulable proteins, contained practically no sugar—hence glucose as such was not stored in the liver. The aqueous extract, however, was opalescent, and he found subsequently that this opalescence was due to the presence of a substance precipitable by alcohol, to which he gave the name of *glycogen*, *i.e.* sugar-former, because from it glucose could be readily obtained. The large amount of sugar found in the liver which had been kept warm after removal was due to conversion of the glycogen into glucose by the action of enzymes present in the liver.‡ That it is an enzyme action is proved by the fact that fresh liver may be dehydrated with alcohol, dried and powdered, and kept for months in this condition without any alteration occurring in its glycogen. If, however, the dried powdered liver be moistened and allowed to remain at the temperature of the body for some hours, its glycogen disappears and the equivalent quantity of glucose takes its place; this change does not occur if the liver is first boiled.

In the past the conversion of glycogen to glucose was attributed to a single enzyme called glycogenase.

We now know that in the conversion two enzymes are concerned, viz., a *phosphorylase* and a *phosphatase*.§ The phosphorylase, in presence of its co-enzyme, adenylic acid, catalyses the reaction :—

$$\text{glycogen} + H_3PO_4 \rightleftarrows \text{glucose-1-phosphate}$$

while the phosphatase catalyses the reaction.

$$\text{glucose-1-phosphate} \rightleftarrows \text{glucose} + H_3PO_4.$$

By reversal of the first reaction, glycogen can be produced from glucose-1-phosphate.‖

Glycogen is a white, tasteless and odourless polysaccharide of the empirical formula $C_6H_{10}O_5$. Like starch, it resists the action of boiling potash,¶ but is hydrolysed by acids, or by amylolytic enzymes, into dextrins,

* BERNARD. *Mém. Soc. Biol.*, 1849, 121; *Compt. Rend.*, 1850, **21**, 571.
† BERNARD. *Compt. Rend.*, 1855, **41**, 461.
‡ HODGSON. *Biochem. J.*, 1936, **30**, 542.
§ CORI. *Endocrinol.*, 1940, **26**, 285.
‖ CORI, CORI and SCHMIDT, 1939, *J. Biol. Chem.*, **129**, 629.
¶ BERNARD. *Compt. Rend.*, 1857, **44**, 578.

maltose and finally glucose. Its aqueous solution with iodine gives a brownish red colour, and it is precipitated by 55 per cent. alcohol, strong acetic acid, and various other precipitants which abstract water.

Glycogen may be estimated by Pflüger's method * of which there are many modifications; the principle of the method is that weighed samples of the tissue are dropped into their own volume of 60 per cent. boiling potash solution and then heated on a water-bath for one to four hours. This dissolves practically everything in the tissue; alcohol is then added in amount to make up to 70 per cent.; the precipitated glycogen is dissolved in water, reprecipitated by alcohol, and finally hydrolysed for three hours in 2·2 per cent. HCl. The glucose thus formed is then estimated, and the results usually expressed in terms of glucose.

In order to obtain a large amount of glycogen from the liver, the animal is fed for twelve to twenty-four hours previously on a diet rich in carbohydrates; glycogen may then be present in amount up to 12 per cent. or more of the weight of the fresh liver. Glycogen can also be formed from the proteins of the food or from the products of their disintegration, the amino-acids and the liver can continue to supply sugar to the blood on a diet which includes only proteins and fats; in such animals glycogen itself can be stored up at the expense of protein. It is doubtful whether a perfectly normal existence is possible in man in the total absence of carbohydrates from the food, though in the northern nations, *e.g.* the Eskimos, the amount of carbohydrate consumed is very small in comparison with the fats and proteins. It does not seem to be possible for the liver to *store* glycogen out of fats. At any rate, that is the interpretation which is generally placed on experiments on feeding with fats. In these experiments it is found that if fats be administered to an animal after the liver has been nearly freed from glycogen, although the liver may store up fats, it does not store up any glycogen. This may be because fat is only slowly converted, so that all the glucose formed from it would be required for use forthwith instead of being stored as glycogen.

The glycogen of the liver represents a reserve material. If an animal be starved, the glycogen quickly diminishes in the liver, although, even at the end of ten or twelve days' complete deprivation of food, small traces of glycogen may still be found in this organ. If starvation be combined with hard work, the liver glycogen is lowered more quickly. The same reduction of glycogen may be produced by any means which evoke an increased muscular activity, *e.g.* in strychnine convulsions. Of the various reserve materials which are available, the carbohydrate is the first to be called upon to meet the increased needs of the tissues during functional activity, such as muscular work or greater heat production. Thus the glycogen is rapidly reduced in the liver of a rabbit which has been immersed in a cold bath.

On account of the importance of glycogen as a reserve material, it is produced and stored up in almost all growing tissues, to be utilised in their subsequent development. Thus it is found in large quantities in the placenta during a certain period, in fœtal muscles, in yeast, in oysters, and in the muscles of the body generally. In fœtal muscles it may amount to as much as 40 per cent. of the total dried solids.

It is principally to Claude Bernard that we owe the conception of the glycogenic function of the liver which is still current. According to this, the liver cells continually form glycogen, which they can manufacture from

* PFLÜGER. Art. "Glycogène" in *Richet's Dictionnaire de Physiol.*, 1907, Vol. [G]228.

a variety of materials. The glycogen so formed may either remain and accumulate in the liver cells, or it may be converted into glucose and pass out into the blood. Both processes, manufacture of glycogen and conversion of glycogen to glucose, are usually in operation together; if glycogen formation predominates over breakdown, the glycogen store in the liver increases, if the formation of glucose predominates, the store is depleted and formation of glucose ultimately slows down. The balance between the formation and breakdown of glycogen in the liver is determined, on the one hand by the quantity of carbohydrate available to the body, whether derived from ingested carbohydrate or from glucose produced from protein or fat, and on the other hand by the demand for carbohydrate by the various tissues of the body.

In fasting, the blood will have a lowered content of glucose. The liver now, by the activity of its enzymes, rapidly converts its glycogen back

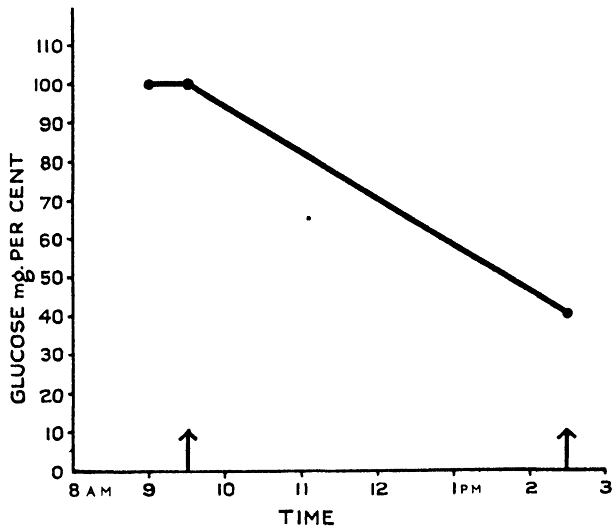

FIG. 572. Blood glucose concentrations in hepatectomised dogs (average of 12 expts.) Liver removed at first arrow; animal moribund, with hypoglycæmic sugar level at second arrow, five hours later. (MANN and MAGATH, 1923.)

into glucose—a process called *glycogenolysis*—and this glucose is carried away in the blood of the hepatic veins which contains much more glucose than that in the portal vein or general ciculation. In this way, the liver acts as a storehouse, in which carbohydrate can be temporarily stored and brought out as required.

The liver is giving out glucose to the blood most of the time, though not always at the same rate. After removal of the liver the blood sugar rapidly falls (Fig. 572), but this may be prevented by constant infusion of glucose at a proper rate; when the liver is present the glucose of the blood is kept up during fasting by conversion of liver glycogen to glucose at the same rate. When a carbohydrate meal is given, absorbed glucose enters the portal blood; the liver does not now add so much glucose to the blood, and if absorption is rapid enough it may add none at all. In fact, if the blood sugar is greatly raised, as by intravenous injection of glucose, the liver may actually take up sugar from the blood (Fig. 573), though this does not easily occur. How this regulating action of the liver is effected it is at present impossible

to say. After carbohydrate feeding, glycogen accumulates rapidly in the liver, and this has led to the belief that the absorbed carbohydrate has been directly stored in the liver as glycogen. But that this simple explanation does not suffice is shown by the fact that rarely, if ever, does the hepatic vein blood contain less sugar than the portal blood. Nearly always it contains more, though the excess is much smaller during the absorption of a carbohydrate meal.* One substance, viz. lactic acid, is, however, removed from the blood as it passes from the portal vein through the liver, and this does seem to be converted by the liver into glycogen.

When a liver is perfused with blood containing glucose, no glycogen is deposited, but if lactic acid, or fructose, is added instead of glucose, then this is taken up and glycogen is formed.† Probably, therefore, glucose must first be converted to lactic acid before the liver can form glycogen from it. The liver, however, can, and does, convert other things than lactic acid into glycogen; thus it is certain that it can convert pyruvic acid,

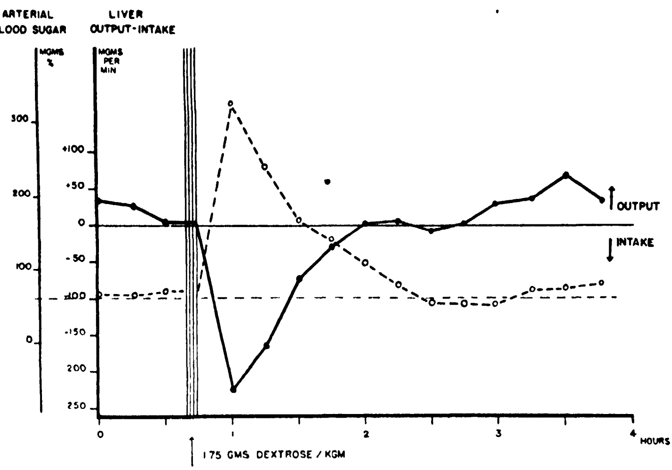

FIG. 573. Arterial blood sugar concentration (discontinuous line), and liver output or intake of sugar (continuous line) before and after intravenous injection of glucose Except when arterial sugar is high, the liver is giving out sugar. (SOSKIN *et al*, 1939)

glycerol, dihydroxyacetone, methyl glyoxal, and some amino-acids into glycogen. Hence the liver glycogen may not only be formed after carbohydrate ingestion, but it may also arise from protein and perhaps from fat, by synthetic processes which occur in the liver. In starvation the available carbohydrate would soon be used up, but new formation of carbohydrate, or *glyconeogenesis*, from protein and fat supplies the constant demand for blood sugar.

The glycogen content of the liver, therefore, represents the balance between glycogen formation from these various sources, and glycogen breakdown to glucose, and it is probable that both these processes are continually in operation.

There are various ways by which the process of glycogen storage in the liver may be studied. One of the simplest and most direct of these is the method introduced by

* TSAI and YI. *Chinese. J. Physiol.*, 1936, **10**, 105.
† LUNDEGAARD, NIELSEN and ØRSKOV. *Skand. arch. Physiol.*, 1936, **13**, 296.

Cori and Cori.* They showed that the livers of different white rats of a standard, inbred strain, contain equal amounts of glycogen when the animals are all treated identically. If the animals are allowed to fast for twenty-four hours, their livers lose considerably in weight, and the liver glycogen is reduced to about 0 2 to 0 5 per cent. When a sugar solution, *e.g.* glucose, is given in sufficient amount, it is quickly absorbed, and at a constant rate which, as we have already learnt, is characteristic, not of the concentration of the solution given, but of the variety of the sugar. Thus, three batches of fasted rats may be taken ; to the first is given by stomach tube some water, to the second the same amount of water containing glucose, and to the third water and a similar weight of fructose. Then one or more of the animals in each batch is killed each hour and the glycogen in its liver determined, the water-fed animals serving as controls. In this way the rate of glycogen formation after ingestion of these sugars can be demonstrated. It was found that when 1 to 2 grammes of sugar was given per 100 grammes of body weight, the maximum amount of liver glycogen was formed after four hours. After glucose the liver contained 5·3 per cent., and after fructose 5 7 per cent. glycogen. Different sugars, however, are absorbed at different rates, *e.g.* in the four hours 1 06 grammes of the glucose and 0 540 gramme of the fructose was absorbed per 100 grammes of rat, and when the glycogen formation is recalculated, taking this into account, it was found that in four hours the amounts of glycogen deposited represented 18 per cent. of the absorbed glucose and 38 per cent. of the absorbed fructose.

The rate at which glycogen is formed in the liver depends to some extent on the concentration of sugar in the blood , the higher the blood sugar the less need there is for the liver to supply it, and the greater the amount of glycogen retained in the liver. It is often assumed, when glucose is given and the liver glycogen is increased, that this increase represents storage as glycogen, by the liver, of the absorbed glucose. This is certainly only partly true ; some of the accumulated liver glycogen arises by processes of glyconeogenesis and is retained by the liver, while the absorbed glucose is used by the tissues, instead of drawing upon liver glycogen.

GLYCOGEN STORAGE IN MUSCLE. Skeletal muscle contains from 0·05 to 1·0 per cent. of glycogen, which is formed from the blood sugar. The deposition of glycogen is probably always going on to replace the glycogen continually being used up by the muscle. Hence the venous blood going away from a muscle contains definitely less glucose than the arterial blood supplied to it. Though normally muscle can take up blood sugar and convert it into glycogen, this process happens much more rapidly when insulin, the hormone derived from the pancreas, is present in excess in the circulating blood. Although, as we have already seen, the muscles can transform *d*-lactic acid into glycogen, they appear able to do this much less rapidly than the liver, so that if lactic acid is set free into the circulation from the muscles during muscular exercise, it is probably mostly converted into glycogen by the liver ; the muscles then restore their glycogen from the blood sugar, which is replenished either by absorbed glucose from the alimentary canal or by breakdown of liver glycogen.

Though the muscles contain a smaller percentage of glycogen than is present in the liver, the total amount of muscle in the body is so great that the whole musculature usually stores much more glycogen than the liver. Thus, in a well-fed man of average weight the liver can perhaps hold 200 grammes of glycogen, while the muscles might hold 350 grammes of glycogen ; in the fasting individual the muscle glycogen would not be very greatly reduced, whereas the total liver glycogen might be as low as 10 or 20 grammes. It is evident, therefore, that the liver glycogen is much more mobile than the muscle glycogen, and even if the latter is considerably reduced during exercise it is soon replenished, largely at the expense of the liver glycogen.

* Cori and Cori. *J. Biol. Chem.*, 1926, **70**, 577.

WITHDRAWAL OF GLYCOGEN FROM LIVER AND MUSCLES. It is a curious fact that glycogen is removed from liver and muscles in different forms; it leaves the liver in the form of glucose, and the muscles in the form of lactic acid, and these two products may enter the blood stream. The explanation is that different enzyme systems are concerned in the two cases. In the liver, as we have seen, there are two enzymes, which between them hydrolyse the glycogen to glucose. In muscle, on the other hand, we have to deal with the complex system, already described in connection with muscular contraction, part of which are enzymes capable of converting the glycogen into lactic acid.

The liver, then, can convert lactic acid and other substances, including no doubt, glucose, into glycogen, and glycogen then into glucose; muscle can convert glucose (or lactic acid) into glycogen, and the glycogen into lactic

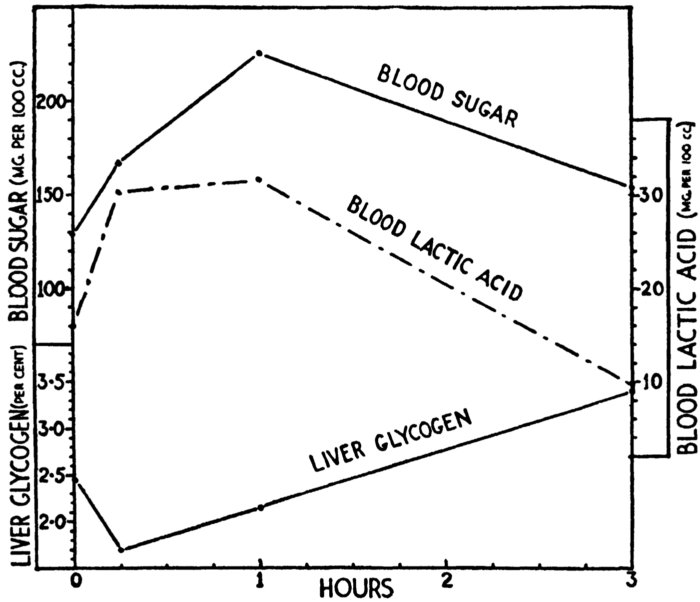

Fig. 574. Effect of subcutaneous injection of adrenaline on blood sugar, blood lactate and liver glycogen of the rat. Adrenaline 0·02 mg./100 grammes given at 0. (Thorpe's *Biochemistry*, from data by Cori and Cori, *J. Biol. Chem.*, 1930, **86**, 375.)

acid. These specialised conversions render possible an interesting cycle of changes, by means of which muscle and liver glycogen become virtually interchangeable in the body. This is seen most clearly in the action of adrenaline in certain animals, such as the rat. If rats fasted for twenty-four hours be given a subcutaneous injection of 0·2 mg. adrenaline per 100 grammes of body weight, they may excrete more glucose than could have been derived from the liver glycogen. Yet it is found that the liver glycogen at the end of three hours has *risen* from an average of 6 mg. per 100 grammes of body weight to 42 mg. per 100 grammes body weight, but the muscle glycogen has *fallen* from 128 mg. to 71 mg. per 100 grammes body weight.* What happens under these conditions is that the adrenaline for some reason causes conversion of muscle glycogen to lactic acid, the concentration of which in the blood is at first considerably increased; the lactic acid is then

* Cori and Cori. *J. Biol. Chem.*, 1928, **79**, 309.

taken up by the liver and converted into liver glycogen (Fig. 574). This important cycle, which may conveniently be called "the Cori cycle," is represented thus :—

$$\begin{tikzcd} & \text{Blood glucose} \arrow[dr] & \\ \text{Liver glycogen} \arrow[ur] & & \text{Muscle glycogen} \arrow[dl] \\ & \text{Blood lactic acid} \arrow[ul] & \end{tikzcd}$$

After sixteen hours the increased liver glycogen has disappeared again. The principal conditions under which glycogen is withdrawn from muscle (as lactic acid) are under the action of severe exercise and adrenaline.

The liver converts its glycogen into glucose under a great variety of conditions, most of which can be brought together as being due to the action upon the liver, either of nervous influences, or of adrenaline circulating in the blood. We may suppose, as a convenient hypothesis, that a breakdown of liver glycogen by the action of adrenaline or by nerve stimulation may be inhibited by the action of insulin. This does not imply, however, that insulin by itself might be able to lead to formation of liver glycogen.

The blood sugar can arise directly from two sources only, viz., from absorbed carbohydrate and from liver glycogen. In the fasting animal the only direct source of blood sugar is the glycogen of the liver. This is shown by the fact that after removal of the liver the blood sugar steadily falls, and the animal soon passes into hypoglycæmic convulsions unless it receives frequent intravenous injections of glucose.

HYPERGLYCÆMIA AND GLYCOSURIA

Normal urine contains a small proportion of reducing substance, but at least half of this is not glucose. The term *glycosuria* is not employed unless glucose appears in quantities large enough to give a reaction with Fehling's solution. Such a condition may easily be brought about by the injection of glucose, subcutaneously or intravenously. Cane sugar or lactose, introduced in the circulation, is quantitatively excreted in the urine. A rather larger quantity of maltose may be injected slowly without appearing in the urine, since the blood serum contains maltase, which converts the maltose into glucose. Glucose, fructose, mannose or galactose, if introduced slowly enough into the circulation, are used up, and glycogen appears in the liver. If, however, the sugar in the blood rises above about 0·18 per cent. (the *renal sugar threshold*), glucose appears in the urine.

The occurrence of glycosuria depends upon two things: (1) the sugar content of the blood; (2) the renal sugar threshold. When glycosuria occurs it means that the blood sugar has risen to a value higher than the renal threshold (normally 180 mg./100 c.c.); consequently, glycosurias may be due to one of two classes of cause: first, to an abnormally high blood sugar; second, to an abnormally low kidney sugar threshold. The latter type, occurring naturally, is called *renal glycosuria*, and has no further interest for

us here, apart from the importance of distinguishing between it and other forms which are more serious.

(1) **ALIMENTARY GLYCOSURIA.** A state of hyperglycæmia may be induced by the administration of abnormally large quantities of glucose by the mouth. Glycosuria from this cause in normal people, however, is largely a figment of the imagination, since the body can dispose of the excess of glucose as fast as it is absorbed into the blood stream; even when 500 grammes of glucose is taken no sugar may be passed, or only fractions of a gramme.* In certain individuals the power of utilising glucose may be deficient, so that prolonged hyperglycæmia and copious glycosuria may be caused by any over-indulgence in carbohydrate food. This condition is revealed by a glucose tolerance test.

(2) **INFLUENCE OF THE NERVOUS SYSTEM.** The liver is very richly supplied with nerves, which are derived principally from the sympathetic system. Division of these nerves does not seem to affect the glycogenic function of the liver.† Stimulation of them, however, or stimulation of the splanchnic nerves (after removal of the suprarenals), produces rapid reduction in the glycogen content of the liver, hyperglycæmia and glycosuria. The effects are greater when the splanchnic nerves are stimulated than when the liver nerves are stimulated direct. This is probably because adrenaline-like substances are set free from other tissues besides the suprarenal medulla.

(3) **THE EFFECT OF ADRENALINE.** The injection of small amounts of adrenaline into the circulation has a similar effect to that of stimulation of the splanchnic nerves; in fact, much of the effect of stimulation of the splanchnics is certainly due to the liberation of adrenaline into the circulating blood which is caused thereby. Hence, if the nerves going to the liver are divided, stimulation of the splanchnics will still cause depletion of liver glycogen, hyperglycæmia and glycosuria, but if the suprarenals are also removed, then stimulation of the splanchnics is without much effect.

Adrenaline has been said by some to reduce,‡ and by others to increase § the utilisation, by oxidation, of glucose by the tissues; the evidence cannot be discussed here.

Asphyxia, the administration of anæsthetics, infective processes, pain, emotion, all cause depletion of liver glycogen, usually with a rise of blood sugar, and often with glycosuria. Most of these factors act by causing a discharge of impulses along the sympathetic nervous system, which include among their effects a liberation of adrenaline into the circulating blood. If the suprarenals are previously removed, these conditions cause a smaller rise of blood sugar and smaller loss of liver glycogen. If the liver is removed no rise of blood sugar is caused in these conditions.

Prolonged administration of adrenaline, as by subcutaneous administration, may give results which at first appear to differ from those of short intravenous injections. This is because with more protracted dosage the Cori cycle becomes increasingly important, with the result that muscle glycogen is converted first into lactic acid and then into liver glycogen, which at the end may be raised.

(4) **PIQÛRE GLYCOSURIA.** An important cause of glycosuria is that due to certain injuries of the central nervous system. It was shown

* TAYLOR and HULTON. *J. Biol. Chem.*, 1916, **25**, 173; BENNETT and DODDS. *J. Physiol.*, 1925, **59**, Proc. lxiii.

† DONALD. *Amer. J. Physiol.*, 1931, **98**, 605.

‡ CORI. *Physiol. Rev.*, 1931, **11**, 143.

§ HIMSWORTH and SCOTT. *J. Physiol.*, 1938, **93**, 159

by Claude Bernard,* using rabbits, that puncture at a certain spot on the floor of the fourth ventricle was immediately followed by glycosuria. This piqûre or puncture glycosuria may last for twenty-four hours or more, and is accompanied by hyperglycæmia, the blood sugar level reaching 300-400 mg./100 c.c. at the height of the glycosuria. The hyperglycæmia and glycosuria are more evident if the animal has previously been well fed, but are still present even in starved animals.

The hyperglycæmia is due to the stimulation of a group of nerve cells from which impulses pass out by way of the splanchnic nerves to the suprarenals and liver. In support of this opinion it is found that if both splanchnic nerves have previously been divided the hyperglycæmia and glycosuria are much reduced or altogether lacking. The "centre" can be reflexly affected by stimulation of various afferent nerves, such as the vagus, sensory nerves, &c.

Effects somewhat similar are seen when the central nervous system is transected at certain levels; thus in cats, section across the midbrain at the level of the superior corpora quadrigemina, is followed by prolonged hyperglycæmia and glycosuria.† The same condition is seen on removal of the cerebral cortex,‡ or section of the hypothalamus,§ after which operations there is a sustained excitation of the whole sympathetic nervous system. In the rabbit, however, it seems that decerebration through the pons or piqûre of the floor of the fourth ventricle at the level of the pons is the only central nervous lesion capable of producing this condition.||

It would be easy to suppose that the large sympathetic outflow, accompanied by the constant presence of adrenaline in the circulating blood, caused a rapid conversion of the stored glycogen of the liver into blood sugar, and that this offered a satisfactory and complete explanation for all the events in carbohydrate metabolism following piqûre. In all probability, however, such a simple explanation would not be adequate to meet all the facts. Thus, piqûre or decerebration causes a large out-pouring of sugar into the blood, even in fasting animals, and in these the amount of sugar released may be greater than can be accounted for from the observed reduction of glycogen in liver and muscles.¶ Indeed, in fasting animals in which the liver glycogen is initially low, piqûre or decerebration may even produce an increase of liver glycogen, in spite of the large amount of sugar which is excreted. It would seem probable, therefore, that the consequences of piqûre may be three-fold: first, in the well-fed animal a discharge of liver glycogen in the form of excreted glucose; second, transfer (often only transitorily) of muscle glycogen to the liver, by way of the Cori cycle; third, in the fasting animal, in addition to the discharge of glycogen from the liver, processes of glyconeogenesis are set into action, in which glycogen is manufactured in the liver from non-carbohydrate materials.

(5) **PHLORRHIZIN GLYCOSURIA.** Phlorrhizin is a glucoside extracted from the root cortex of the apple tree. It may be hydrolysed into glucose and phloretin. When phlorrhizin or phloretin is administered subcutaneously, it gives rise to intense glycosuria. It is usually given to dogs by subcutaneous injection in doses of 1 gram per day, suspended in olive oil. Certain other glucosides, *e.g.*, arbutin, have similar effects. The glycosuria induced in this way differs from the forms already described, in the fact that it is not due to hyperglycæmia. The blood sugar is, in fact, diminished. In this respect, therefore, the condition resembles renal glycosuria, and only differs from it in degree inasmuch as the renal threshold is here abolished.

* C. BERNARD. "Leçons de Physiologie expérimentale" (Paris), 1855, p. 296; "Système nerveux" (Paris), 1857, p. 397.

† J. MELLANBY. *J. Physiol.*, 1919, **53**, 1.

‡ BULATAO and CANNON. *Amer. J. Physiol.*, 1925, **72**, 295.

§ BEATTIE, BROW and LONG. *Proc. Roy. Soc.*, 1930, **106B**, 253.

|| DONHOFFER and MACLEOD. *Proc. Roy. Soc.*, 1932, **110B**, 125.

¶ DONHOFFER and MACLEOD, *Proc. Roy Soc.*, 1932, **110B**, 141, 158; EVANS, TSAI and YOUNG, *J. Physiol.*, 1931, **73**, 81.

The action of phlorrhizin is due to the fact that it poisons the enzyme phosphatase. This enzyme is responsible for converting glucose into hexose phosphate and is present in high concentration in the kidney. Glucose cannot be reabsorbed by the kidney tubules except in the form of hexose phosphate and so is allowed to escape into the urine instead of being returned to the blood as in the normal kidney.* If cannulæ be placed in the two ureters so as to collect the urine from each kidney separately, and a small dose of phlorrhizin be then injected into the left renal artery, the urine flowing from the left ureter will in two minutes be found to contain sugar, while the urine from the right kidney will not contain any sugar for another five or ten minutes.

The effect of phlorrhizin is to continually drain off sugar from the blood, and the various effects which it produces are consequences of this. As some of the effects of phlorrhizin poisoning closely resemble the phenomenon of diabetes mellitus, it was once thought that the fundamental causes were similar, but this is no longer believed. It might be expected that the low blood sugar which results from administration of phlorrhizin might induce the effects characteristic of hypoglycæmia, but this is not so for two reasons : first, because the blood sugar does not fall sufficiently as a rule ; secondly, because the tissue sugar is not so greatly reduced as the blood sugar. A further point is that the ability of the tissues to utilise such sugar as they can get, and of the liver and muscles to form glycogen, is not abolished.

In order to maintain the glucose content of the blood at its normal height, the liver must produce fresh sugar to take the place of that lost by the kidneys. At first the liver will convert most of its stored-up glucogen. If a dose of phlorrhizin be given to each of two animals and one animal be killed as soon as the excretion of sugar slows down, the liver will be found poor in glycogen. If now a second dose of phlorrhizin be given to the other, which may now be regarded as nearly free from glycogen, glycosuria is produced as before, and the excretion of sugar can be continued indefinitely by repeated administration of the drug.

So long as sufficient carbohydrate food is given, the loss of sugar does not entail any increase in the destruction of the tissues ; but otherwise the waste of sugar has to be made good at the expense of material other than carbohydrate. The readiest source of the sugar excreted by a starved phlorrhizinised animal is the tissue protein. The nitrogen excreted in the urine in fasting phlorrhizinised animals varies in proportion to the quantity of sugar excreted, and there is a constant ratio between the amounts of nitrogen and of sugar excreted. In different experiments this ratio G : N varies from 2·8 : 1 to 3·6 : 1. These effects in phlorrhizin glycosuria are shown in the following Table (Lusk) :—

	Goat			Dog		
	G	N	G : N	G	N	G : N
Fasting	—	3·72	—	—	4·04	—
Fasting	—	3·71	—	—	4·17	—
Fasting and glycosuric	20·33	4·90	4·15	63·55	12·66	5·02†
Fasting and glycosuric	26·08	8·83	2·95	65·30	18·76	3·38
Fasting and glycosuric	23·39	8·06	2·90	65·84	18·57	3·54
Fasting and glycosuric	19·01	6·84	2·78	64·60	17·29	3·74

* LUNDSGAARD. *Biochem. Ztschr.*, 1933, **264**, 209.

† The high G : N ratio on the first day is due to the conversion of the glycogen still present in the body.

If meat be administered to such starving animals with glycosuria, the G : N ratio does not alter; though the amount of nitrogen in the urine increases, the sugar increases in the same proportion. This is shown in the following respiration experiment (Mandel and Lusk) on a dog with phlorrhizin glycosuria, in which the metabolism during starvation and after ingestion of meat was determined :—

	G : N	Calories from protein	Calories from fat	Calories total.
Fasting . . .	3·69	80·2	274·4	354·6
300 grammes meat .	3·55	161·9	261·7	423·6

Glucose administered to a phlorrhizinised dog is practically all recoverable from the urine.*

The sugar excreted in starvation must be derived from the amino-acids, of the protein. It has been shown by Lusk, Embden, and Dakin that the following amino-acids (now called anti-ketogenic) yield large amounts of glucose when administered to a phlorrhizinised animal: glycine, alanine, serine, cystine, aspartic acid, glutamic acid, ornithine, proline and arginine. We must assume that these amino-acids, produced in digestion or by the autolysis of the tissues, undergo deamination and that the sugar is formed by a process of synthesis from the ketonic acids thereby produced. On the other hand leucine, tyrosine and phenyl alanine (the ketogenic amino-acids) give no increase in the output of sugar. These amino-acids seem to follow the line of fat metabolism, since they are converted into aceto-acetic acid when perfused through a dog's liver. The administration of fats to a phlorrhizinised dog is also without effect on the sugar excretion. This may be because any glyconeogenesis from fat is already proceeding at its maximum rate.

Another effect of continued administration of phlorrhizin is fat infiltration of the liver. This is merely a result of the carbohydrate starvation. The liver seems to be able to act as a storehouse either of fat or of carbohydrate, so that there is an inverse ratio between the amount of glycogen and the amount of fat stored up in the liver at any given time.

The carbohydrate starvation has other deleterious effects, since a certain amount of carbohydrate is a necessary condition for both fat and protein metabolism. The necessity of carbohydrate for the economisation of protein is brought out in an experiment by Cathcart. It has long been known that carbohydrate administration has a sparing effect on protein metabolism. If an animal or man be starved, the nitrogenous output sinks to a certain level and there remains practically stationary. If now pure carbohydrate food be administered, sufficient to meet the energy requirements of the animal or man (about 35 Calories per kilo.), there is at once a rapid drop in the output of nitrogen and therefore in the protein waste of the tissues. Fat has a much slighter or no sparing effect on the nitrogenous metabolism. Indeed in certain experiments by Cathcart the administration of fat caused an actual rise in the nitrogenous output.

Ketosis. The evidence that the carbohydrates play a necessary part in the metabolic history of fats has already been mentioned (*v.* p. 992). We have seen that, in the absence of carbohydrates, the last stages in the oxidation of fats make default, so that the acetone bodies accumulate in large quantities in the blood (*ketonæmia*), and are excreted in the urine (*ketonuria*). The appearance of these keto-acids and acetone is spoken of as *ketosis*, and sugar is said to be *antiketogenic.* The function of sugar

* REILLY, NOLAN and LUSK. *Amer. J. Physiol.*, 1898, **1**, 395.

in this respect may be due to a reaction taking place between one of the 'acetone bodies' and glucose itself or some product of decomposition or oxidation of glucose, such reaction being essential for the complete oxidation of the acetone bodies. This view is supported by the definite relationship between the amounts of carbohydrate and fats which are necessary to prevent ketosis. It has been shown that no ketosis occurs when one molecule of sugar is oxidised in the body for each molecule of the aceto-acetic acid which may be formed from the fats (or a portion of the proteins) of the food. When the proportion is only 1 to 2, there is always some ketosis, and when the proportion of potential aceto-acetic acid to glucose exceeds 2, the whole excess is excreted as ketone bodies. This relationship is of practical importance in the dieting of diabetics.

Shaffer has shown that, when one molecule of sugar is mixed with two molecules of aceto-acetic acid in strongly alkaline solution containing hydrogen peroxide, the whole mixture undergoes rapid and complete oxidation.

Pronounced ketosis occurs in the starving phlorrhizinised animal, but is relieved by the administration of glucose *; this may be either because oxidation of the glucose is not an essential factor in its antiketogenic action, or more probably because phlorrhizin does not abolish the oxidation of glucose in the body.

Not only does the excretion of ketone bodies involve a loss of energy to the body, but these organic acids require bases for their neutralisation, and when these are no longer present in sufficient quantity the acids will be excreted in combination with ammonia. If the condition of carbohydrate starvation be continued, this mechanism of neutralisation is insufficient, and the phenomena of acidosis, viz. dyspnœa and coma, ensue as the death of the animal approaches.

For Reference

LUSK (1928). The Science of Nutrition. (Philadelphia and London.)
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(6) **PANCREATIC DIABETES.** Von Mering and Minkovski† found that pancreatectomy (excision of the pancreas) gives rise to a rapidly fatal diabetes, which is closely similar to the severest cases of diabetes in man. The operation is followed almost at once by hyperglycæmia, and by the appearance of a large amount of sugar, 5 to 10 per cent., in the urine. The glycosuria persists; if glucose is given it is all excreted; there is polyuria and thirst, the urinary nitrogen is raised, and there is sometimes a constant G/N ratio during fasting. The respiratory quotient is low (about 0·7); acetone bodies are excreted; liver and muscle glycogen are much reduced, but the glycogen in the heart is increased; the glucose utilization of the tissues is greatly reduced, as is shown by the slight difference in glucose content between arterial and venous bloods; the animal rapidly wastes, and finally dies at the end of a week or so in diabetic coma. The condition of diabetes observed under these circumstances has nothing to do with the absence of the pancreatic secretion into the intestines, since ligature of the ducts of the pancreas, or grafting the pancreas beneath the skin, does not give rise to diabetes, although these procedures are followed by atrophy of the secretory part of the gland.‡

* WIERZUCHOWSKI. *J. Biol. Chem.*, 1926, **68**, 385.
† VON MERING and MINKOVSKI. *Arch. f. exp. Path.*, 1889, **26**, 371.
‡ SSOBOLEW. *Virchow's Arch.*, 1902, **168**, 91.

We thus see that the pancreas has a two-fold function: the secretion of the pancreatic juice and an influence on general metabolism, the absence of which is followed by diabetes. Corresponding with this two-fold function, two kinds of structures are present in the gland, the secreting acini and the islets of Langerhans. These latter, though arising in connection with the ducts, are solid masses of cells and have no communication with the lumen of the ducts. According to Lane* and Bensley,† the stainable islet cells may be divided into two varieties, which have been given the name of α and β cells, according as their granules are fixed respectively by alcoholic or watery solutions. It has been shown, both by Bensley and by Homans,‡ that these cells undergo no alterations when the gland is excited to secrete by the injection of secretin (Fig. 575). On the other hand, if four-fifths of the pancreas be removed, the remaining part may gradually become inadequate to prevent

A B

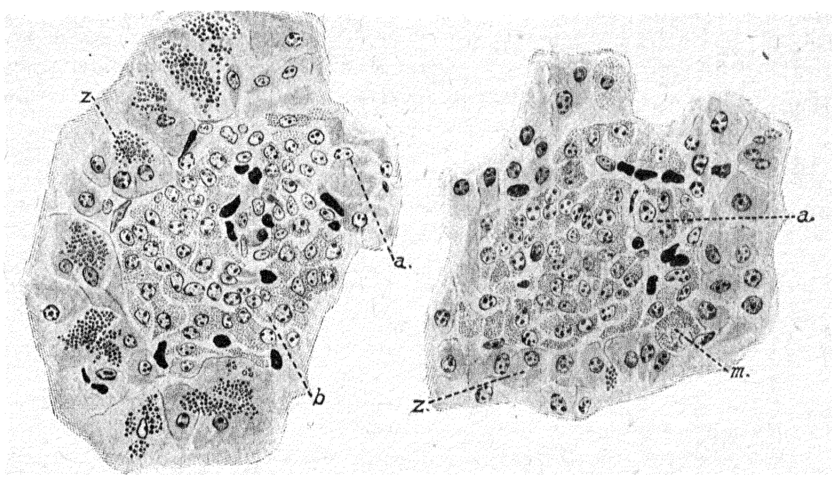

Fig. 575. (A) and (B) show an Islet with the surrounding Tissue in a resting Gland (A) and after Exhaustion with Secretin (B).

In (A) the secreting acini (*z*) are charged with zymogen granules. In (B) these have entirely disappeared. On the other hand no change is noticeable in the cells of the islet. In the latter the granular cells (*m*) are the β cells, and the clear hyaline cells are the α cells. (Homans)

diabetes; and Homans has shown that, when under these circumstances diabetes supervenes, the granules disappear from the β cells. Changes have also been alleged in the islets of Langerhans in fatal cases of diabetes in man.

INSULIN

These facts led physiologists to believe that the pancreas produced in the islets of Langerhans, a hormone necessary for the normal metabolism of carbohydrates. Already in 1916 Schafer had proposed for this hypothetical hormone the name of *insulin*, but it was not till 1922 that Banting and Best § succeeded in extracting the hormone from the normal pancreas, and in show-

* Lane. *Amer. J. Anat.*, 1907, **7**, 409.
† Bensley. *Amer. J. Anat.*, 1911, **12**, 297; *Harvey Lects.*, 1914–15, 250.
‡ Homans. *Proc. Roy. Soc.*, 1913. **86B**, 73.
§ Banting and Best, *J. Lab. Clin. Med.*, 1922, **7**, 251, 464; Banting, Best and Macleod, *Amer. J. Physiol.*, 1922, **59**, 479.

ing that, by its injection, it was possible to obviate the effects of removal of the pancreas in dogs, and by regular dosage to effect a restoration to health in cases of diabetes in man.

In order to prepare insulin, the fresh pancreas is finely minced and extracted with several changes of alcohol which, diluted by the water contained in the gland, is evaporated to a small bulk and poured into absolute alcohol. A white precipitate is produced which represents the crude insulin. The yield of insulin is increased by extracting in the first instance with alcohol containing sodium bicarbonate. Insulin forms an insoluble compound with picric acid, which is decomposed by weak hydrochloric acid with the formation of an hydrochloride. These properties are made use of to effect a further concentration of the active principle.*

The insulin so prepared is a white powder, obtainable in crystalline form,† easily soluble in water and in dilute alcohol. All crystalline insulin contains zinc, and if this is removed the material is not crystallisable.‡ It does not dialyse, will withstand boiling for a short time, is stable in acid solutions, and undergoes slow oxidation in alkaline solution. It is a protein of molecular weight 35,000, contains sulphur, and is destroyed by pepsin and trypsin, so that it cannot be given by mouth. 0·2 milligramme injected into a normal rabbit causes a rapid diminution of the blood sugar, and finally the symptoms of hypoglycæmia already described.

The strength of insulin preparations must be carefully controlled by biological standardisation. The method is based on the reduction of the blood-sugar level of animals by the sample. A dose of about 3 international units will in four hours lower the blood sugar level of a 2 kg. fasting rabbit to the convulsive level (0·045 per cent.). The present method is to determine the fall of blood sugar brought about in five hours in a fasting rabbit by subcutaneous injection of known amounts of the solution to be tested, as compared with that induced by the international standard preparation, of which 1 mg. contains 22 units. The purest crystalline insulin has a potency of about 23 units per mg.

If insulin be injected into a pancreatectomised animal the excretion both of sugar and of ketone bodies ceases, while the respiratory quotient and the arterio-venous glucose difference rise, showing that the animal is utilising sugar; and on the administration of carbohydrates glycogen accumulates in the liver. The animal increases at the same time in weight and strength, and can be maintained alive for its normal span. It is necessary, however, to inject a solution of insulin subcutaneously two or three times a day, since this substance is gradually used up in the body.

One of the first questions concerning the action of insulin is the fate of the blood sugar which so rapidly disappears when insulin is given. In the normal animal the tissue carbohydrate and blood sugar may be regarded as derived either directly from ingested carbohydrate, or indirectly from glycogen formed from the breakdown of protein, and probably to some extent also from fat. Whether carbohydrate is ingested or not, its presence and continual metabolism in the tissues is essential to life. The amount of carbohydrate at the disposal of the body at any time thus depends upon (1) the store present as glucose and glycogen in the blood and tissues; (2) the amount accruing from absorption of carbohydrate; (3) the amount formed by glyconeogenesis.

The excretion of sugar in diabetes is due to hyperglycæmia. Glycogen disappears almost entirely from the liver. This state of hyperglycæmia, and

* DUDLEY, *Bioch. J.*, 1923, **17**, 376; 1924, **18**, 665; DODDS and DICKENS, *Lancet*, 1924 (i), 330; *Brit. J. Exp. Path.*, 1924, **5**, 115.

† HARINGTON and SCOTT. *Bioch. J.*, 1929, **23**, 384.

‡ SCOTT. *Biochem J.*, 1934, **28** 1592.

the excretion of sugar, persist even when the animal is completely starved or is fed on a pure protein or protein *plus* fat diet. Moreover, we find in the starved diabetic a constant ratio between the sugar and the urinary nitrogen, the G : N ratio being usually about 2·8, from which it has been inferred that, in the absence of carbohydrate from the diet, the sugar is derived mainly from proteins.

The metabolism of the diabetic animal is characterised by partial failure of the liver and muscles to store carbohydrate, and further by the *accelerated glyconeogenesis* from protein, and probably also from fat. One of the most striking features of the condition is the rapid diminution of the fat depôts of the body, attended by a condition of lipæmia and accumulation of fat in the liver. One of the first effects of extirpation of the pancreas is therefore a rapid fat mobilisation, and the respiratory quotient agrees with that obtained when the metabolic needs of the body are being largely satisfied at the expense of its fat. The tissues also exhibit a diminished power to burn glucose, as is shown by the low respiratory quotient, by the reduced arterio-venous blood sugar difference, and by direct experiment on surviving tissues. Administration of glucose to the animal causes an almost corresponding increase in the amount of glucose excreted in the urine ; this is probably due to the fact that sugar is already being formed by gluconeogenesis at a rate too fast for the tissues, with their reduced powers of oxidation, to utilise it, together with the failure of the liver and muscles to store glycogen. But though this may be conceded, the power of oxidising sugar is certainly not abolished. With increased production and diminished storage of carbohydrate, the blood is flooded with sugar, which escapes by the kidneys, while the accelerated breakdown of fat leads to the formation of ketone bodies in large amount.

In the diabetic subject, it seems quite clear that the effect of administering suitable amounts of insulin is to restore the carbohydrate metabolism to normal; in other words, to accelerate the combustion of carbohydrate, to check glyconeogenesis, and to allow the liver and muscles to carry a store of glycogen ; hence, to lower the blood sugar ; because the carbohydrate metabolism is restored, and because new formation of carbohydrate is reduced, ketone bodies speedily disappear from the blood, and therefore from the urine and breath. It is evident that, in the diabetic, insulin produces all these effects by supplementing the inadequate action of the pancreas.

In the normal animal or man, there is already an efficiently functioning pancreas, so that by administering insulin to them we shall get abnormal effects due to the presence of an excess of that substance. The effects of this excess of insulin are best understood if we consider them as an exaggeration of the effects of therapeutic doses on the diabetic, *i.e.* as displaying the same qualitative influences as in the normal metabolism of carbohydrate, but to an enhanced degree.

We should therefore expect the following effects to ensue if insulin were given in rather large doses to the normal animal ; first, an increased storage of glycogen in the muscles and liver, together with an accelerated combustion of glucose in the tissues. Secondly, a reduction, to a level far below the normal, of the formation of carbohydrate from protein and from fat. In consequence of both of these effects, there would be a large reduction in the blood-sugar level, with its attendant consequences. One of these consequences is the onset of convulsions ; another is that the liver glycogen is now drawn upon, as will be explained later. How long the liver and muscles will be able to supply carbohydrate to the animal which is now deprived of the normal endogenous new formation of sugar will depend on the total available store of carbohydrate in the body at the time the dose of insulin was given, but it is certain that in fasting animals with rapid metabolism, such as mice, the store is very small. If the mouse were oxidising

carbohydrate exclusively for its metabolism, its oxygen consumption shows that i store would last for only about twenty minutes if new formation from protein a fat ceased. If the action of insulin is that explained above, we should expect find that, by stopping glyconeogenesis and thus confining the animal's metabolism to ready-formed carbohydrate, there would, if no more carbohydrate were supplied, be rapid falling off in the respiratory exchange, accompanied by a fall of body temperatu while if the fall of body temperature were prevented by placing the animal in a th mostat, we should expect that the animal would die in about half an hour, or as so as its carbohydrate was completely exhausted. This is what actually happens, and other species of animals the fatal result depends on similar conditions, and is acco panied by a similar depletion of the body store of carbohydrate.

In the later stages, therefore, the glycogen content of the liver and muscles, inste of being raised, is greatly lowered.*

The emptying of the liver glycogen in consequence of the fall of blood sugar is d to the effects of the lowered blood sugar on the central nervous system, from wh efferent impulses pass to the liver and to the suprarenals *viâ* the sympathetic ner supply. The adrenaline set free from the suprarenals rapidly lowers the liver glycog

We must now consider what happens to the glucose which disappears from the blo when insulin is given. It was suggested by Lesser that part of the glucose was oxidis and, as happens in the oxidation of lactic acid in muscle during recovery, the remain of the glucose was built up into glycogen, and subsequent researches have given go grounds for believing that the explanation lies along some such lines.

Best, Hoet and Marks † showed that when the blood sugar of the eviscerated sp cat was kept at a high level for two to five hours by intravenous injection of gluco there was no demonstrable change in the glycogen content of the skeletal muscl When insulin was also given, there was an accelerated rate of sugar disappearance, a an *increase* in the glycogen content of the muscles, irrespective of whether actual hy glycæmia resulted or not, and this deposition of the glycogen was enough to account from 40 to 50 per cent. of the sugar which had been lost from the blood. It may concluded, then, that under the conditions of these experiments, much of the lost sug was recoverable as glycogen.

Finally, Best, Dale, Hoet and Marks‡ showed that the sugar which disappears fr the body can be completely accounted for on the one hand by combustion and on the ot hand by conversion into muscle glycogen.

The Normal Regulation of Insulin Action. Large amounts carbohydrate can be dealt with by the normal individual without any app ciable loss of sugar by way of the urine ; on the other hand, abstinence fro carbohydrates for quite long periods does not lead to hypoglycæmia. This no doubt due to the fact that protein, and to a less extent fat, can be convert into glucose, and thus supply the imperative needs of the body for carb hydrate. But all the experience of clinicians in the treatment of diabet by insulin has shown that it is necessary to secure a proper balance betwe the amounts of insulin administered and carbohydrate ingested, as oth wise there will be either an excretion of glucose in the urine, or else t unpleasant effects of hypoglycæmia. In the normal functioning of the pa creas, since neither of these effects ensue, there must therefore be some mea by which the amount of insulin liberated from the islet tissue correspon with the amount of carbohydrate to be dealt with.

Apparently, when carbohydrate is taken by the mouth, as in the admin tration of glucose for the purposes of a tolerance test and blood-sugar curv there is an accompanying liberation of insulin to deal with it ; it is often se that in a blood-sugar curve the blood sugar falls very rapidly after its initi rise, and reaches a level slightly below that of the blood in the fasting sta (Fig. 569).

The rapid fall is not because the absorption of the glucose from the alime

* Dudley and Marrian. *Bioch. J.*, 1923, **17**, 435.

† Best, Hoet and Marks. *Proc. Roy. Soc.*, 1926, **100B**, 33.

‡ Best, Dale, Hoet and Marks. *Proc. Roy. Soc.*, 1926, **100B**, 55.

tary canal is at an end ; this is shown by the fact that in the diabetic subject the rapid fall is not seen, the high level persisting even for some hours after the ingestion of glucose which originated it. The fall in the normal subject without doubt occurs because by that time the insulin production has been raised sufficiently to reduce the blood sugar in spite of the continuing sugar absorption. For this reason it is found that if a second test meal of 50 grammes of glucose is given to a normal subject as soon as the blood sugar has reached its low level, there is little or no further rise in the blood sugar. It has even been shown that a transfusion into a diabetic subject of blood taken from a normal individual during the absorption of glucose will lead to a fall in the blood sugar of the patient, just as though insulin had been given.

It is uncertain how this regulation of insulin output by the pancreas is effected. Since it appears to be unaffected by denervation of the pancreas, it would seem to be

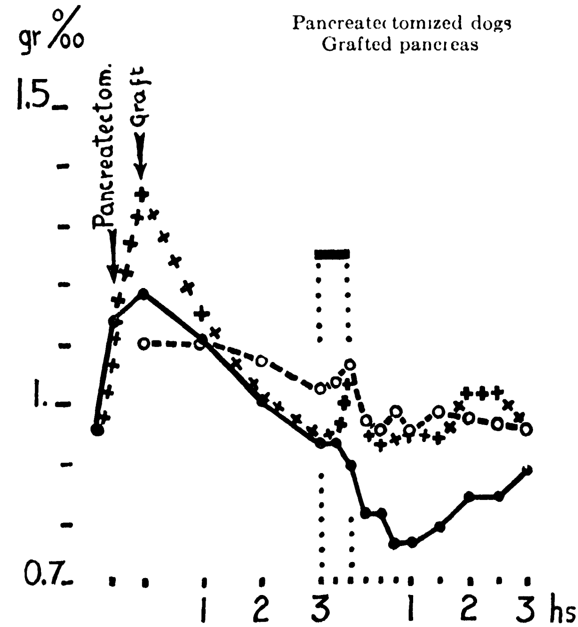

FIG. 576. Injections, at signal, into artery of transplanted pancreas in dogs. ●—● of 25 per cent. glucose +-+-+ of Locke's solution. As a control O - - - O shows effect of injection of glucose solution into jugular vein. (HOUSSAY, after FOGLIA and FERNANDES, 1935.)

independent of any nervous control, and to depend upon a direct effect of the blood sugar level on the pancreas. This is well seen in experiments in which the pancreas has been transplanted, and therefore denervated. When glucose solution is injected into the artery supplying such a pancreas, there is a definite fall in the sugar in the circulating blood (Fig. 576). But Macleod and collaborators and G. A. Clark * have given some evidence that the liberation of insulin can also be controlled by influences reaching the pancreas by way of the vagus.

Nervous control does come into the picture when hypoglycæmia is present. Just as the hyperglycæmia produced by adrenaline, or in any way, can be counteracted by insulin, so the hypoglycæmia brought about by an overdose of insulin can be relieved by administration of adrenaline if sufficient glycogen is present in the liver.

Cannon, McIver and Bliss † have shown that when insulin is given, there is excitation of the sympathetic system and a discharge of adrenaline from the suprarenals as soon as

* G. A. CLARK. *J. Physiol.*, 1926, **61**, 13, 576 ; 1927, **64**, 229 ; 1931, **73**, 297.
† CANNON, MCIVER and BLISS. *Amer. J. Physiol.*, 1924, **69**, 46.

the blood sugar falls below about 0·07 per cent., which checks the further fall of blood sugar; conversely it is likely that the injection of adrenaline into the circulation leads to a setting free of insulin from the pancreas.

(7) INFLUENCE OF ANTERIOR PITUITARY HORMONE (see also Ch. L.). In diseases associated with enlargement of the anterior lobe of the pituitary body (*e.g.* acromegaly and pituitary basophilism) there is in 20 or 30 per cent. of cases, hyperglycæmia and glycosuria.

The work of Houssay and his pupils has shown that the anterior pituitary body produces hormones which have fundamental importance in the regulation of carbohydrate metabolism. The subject is at present in a stage of development, but fundamental results have already been reached.

Hypophysectomy. After hypophysectomy (removal of the pituitary body), no noteworthy effects are seen if the animals are well fed, but on fasting, or on any treatment that lowers the glycogen stores of the body, the blood sugar falls to a low level, and hypoglycæmic seizures ensue. The animals when fasting are demonstrably much (often 30 times) more sensitive to the action of insulin than are normal animals,* and less responsive to adrenaline. Their nitrogen excretion is much below normal.

Combined Pancreatectomy and Hypophysectomy. The results of removal of both pancreas and pituitary are most striking: † the state of diabetes, which usually follows the extirpation of the pancreas is held in abeyance. Animals subjected to the double extirpation are often called ' Houssay ' animals: they show practically normal blood sugars, little if any glucose excretion, no ketosis, normal glucose tolerance, and normal glycogen stores in liver and muscles. Their nitrogen excretion is about normal, and they survive for weeks or months, but are subject to hypoglycæmic crises if subjected to fasting. It seems evident that their carbohydrate metabolism is unstable and is easily tipped in the direction of the hypoglycæmic state.

Injection of Pituitary Extracts. The number of hormones now claimed to be formed by the pituitary anterior lobe is considerable, and there is some doubt about the individual existence of some of them. But by the injection of extracts of the anterior pituitary, prepared in various ways, at least two types of result seem to be clearly established; these are attributed respectively to a diabetogenic and a glycotropic hormone.

The Diabetogenic Hormone. By repeated intraperitoneal injections of extracts of the anterior pituitary into dogs, symptoms resembling those of diabetes develop after a few days: these symptoms comprise hyperglycæmia, glycosuria, ketonuria, high nitrogen output, etc. Extirpation of the liver prevents these results. Given to ' Houssay ' animals, the injections cause a revealing of all the diabetic symptoms.

Usually the symptoms resulting from the injections subside soon after discontinuance of the dosage. But extracts can be prepared which lead, after repeated dosage, to the permanent establishment of a condition closely resembling diabetes.‡ In animals thus rendered permanently glycosuric, the islets of Langerhans were found to have undergone degenerative changes.§

The Glycotropic Factor. Other extracts of anterior pituitary, usually associated, but not identical, with the lactogenic factor (Chap. L.), produce somewhat different results. These do not cause hyperglycæmia nor glyco-

* CORKILL, MARKS and WHITE. *J. Physiol.*, 1933, **80**, 193.
† HOUSSAY and BIASOTTI. *Endocrinol.*, 1931, **15**, 511.
‡ YOUNG. *Lancet*, 1937, *i.*, 372.
§ RICHARDSON and YOUNG. *Lancet*, 1938, *i.*, 1098.

suria, but reduce the sensitivity of the animal to insulin and raise it to adrenaline (Fig. 577).

Injected into hypophysectomised animals, the sugar tolerance is reduced, *i.e.*, a given injection of glucose now causes a longer-lasting rise in blood sugar than in the normal animal (Fig. 578). This is believed to be due to a

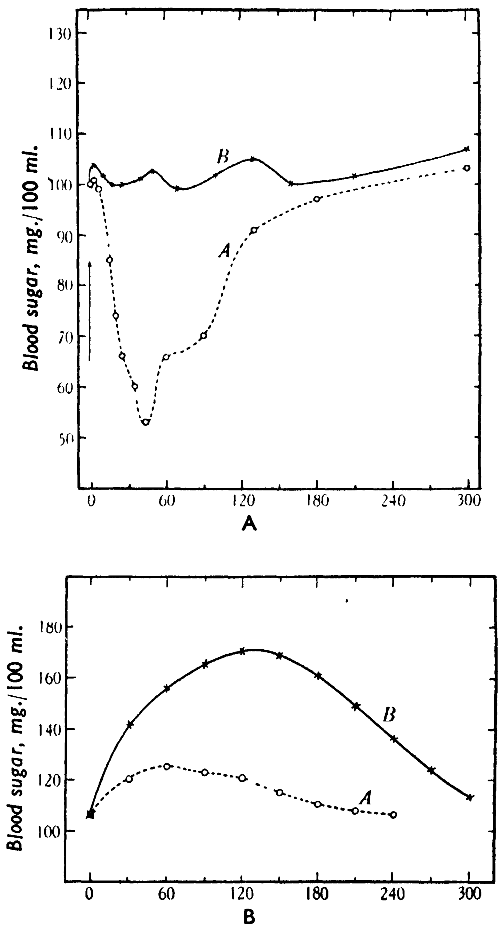

FIG. 577. Effect of anterior pituitary extract on the sensitivity of rabbits to insulin (Graph A) and adrenaline (Graph B).

A. Normal rabbits. B. Rabbits previously treated with anterior pituitary extract. (YOUNG, *Bioch. J.*, 1938, **32**, 1521.)

counteracting of the peripheral action of insulin, so that withdrawal of glucose from the blood by the tissues is diminished.

Probable Function of Anterior Pituitary in Carbohydrate Metabolism. When the complicated relations of the anterior pituitary to carbohydrate metabolism are reviewed, the most probable explanation of the facts seems to be that the hormones of the pituitary exert an influence in favouring glyconeogenesis, in opposition to insulin, which appears to check it. Also, they counteract the peripheral action of insulin on the tissues, and hence reduce the uptake of sugar from the blood. After hypophysectomy, the nitrogen output is reduced, while administration of pituitary extracts

raises it. We may thus suppose that under ordinary conditions, in which the pituitary hormones are present, a good deal of glyconeogenesis is going on, so that, even in starvation, glucose is still being added to the blood by the liver. The administration of insulin to the fasting animal may somewhat tax this supply of carbohydrate, but after hypophysectomy the endogenous supply of carbohydrate is much diminished, while demands by the tissues are not reduced, so that the animals become very much more sensitive to the hypoglycæmic action of insulin. With the action of adrenaline it is the reverse of this—carbohydrate is most available when liver glycogen is high, and labile, so that hypophysectomy reduces the response to adrenaline, and the administration of pituitary extracts enhances

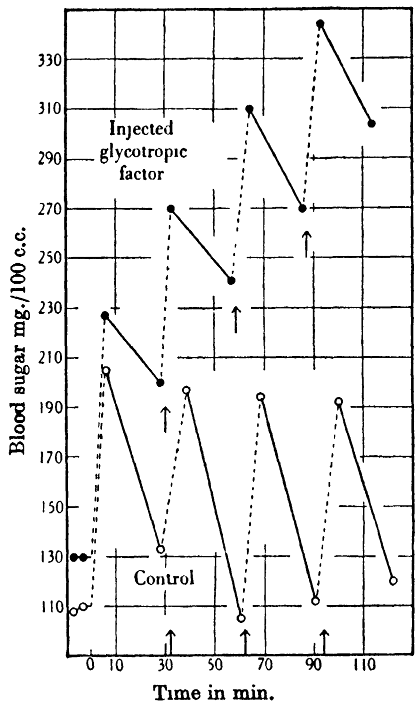

FIG. 578. The effect of half-hourly intravenous injections of 1 gramme glucose on a hypophysectomised rabbit, before and after injections of anterior pituitary extract (Young's glycotropic factor). (HIMSWORTH and SCOTT, *J. Physiol.*, 1938, **91**, 447.)

it. When both pancreas and pituitary are removed, the animal's carbohydrate metabolism somewhat resembles that of the normal state, provided it is well fed and thus given a supply of ready-made carbohydrate to draw upon. Then, although glyconeogenesis is reduced by loss of the pituitary, there is at the same time less tendency to hypoglycæmia because the pancreas is also lacking, and the carbohydrate usage of diabetic tissues not being completely abolished, there is sufficient carbohydrate metabolism going on to meet most ordinary needs.

The amount of carbohydrate consumed in the body is subject to wide variations, dependent on the supply of carbohydrate in the diet. When the supply is restricted, it is endogenously produced from protein and probably also from fat, so that the irreducible minimum of blood sugar is

available as long as life lasts. Determination of the glucose-tolerance curves shows that on low carbohydrate diets the tolerance is reduced and on carbohydrate-rich diets it is augmented, *i.e.*, the more carbohydrate in the diet the more readily can administered glucose be disposed of * (Fig. 579). Hypophysectomy abolishes this effect of diet. Low carbohydrate diet also reduces the response to insulin. All these facts taken together suggest that the pituitary hormones are secreted in greater amount when the diet is poor in carbohydrate and *vice versâ*.† After prolonged total fasting the capacity to utilise ingested sugar is much impaired, so that when sugar is given it

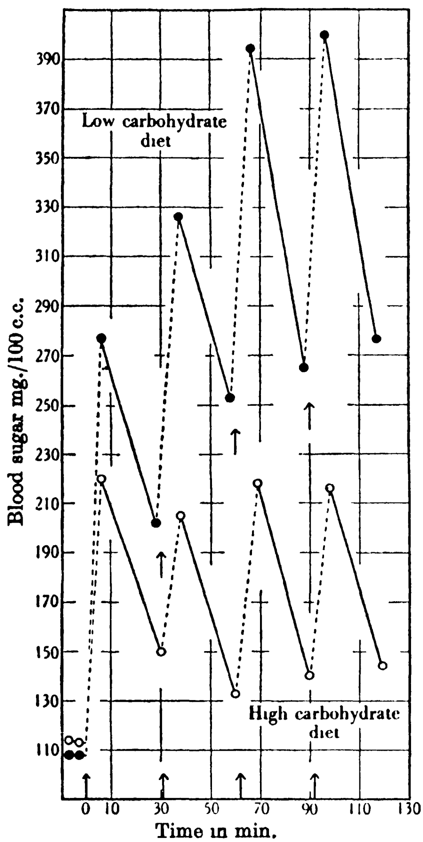

FIG. 579. Response of the same rabbit on high and low carbohydrate diets to half-hourly intravenous injections of 1 gramme glucose. (HIMSWORTH and SCOTT, *J. Physiol*, 1938, **91**, 447)

may at first be excreted unchanged (hunger diabetes). The lost ability to use glucose is not due to inability to store glycogen in the liver ‡ ; it is possibly due to the fact that glyconeogenesis has been going on intensively, the new-formed glycogen being at once converted into glucose, the usage of which is reduced. When glucose is given this floods the organism until such time as the excessive glyconeogenesis and glycogenolysis can be checked and the rate of usage restored.

* HIMSWORTH. *Clin. Sci*, 1935, **2**, 67

† HIMSWORTH and SCOTT, *J. Physiol.*, 1938, **91**, 447.

‡ DANN and CHAMBERS. *J. Biol. Chem.*, 1932, **95**, 413.

INFLUENCE OF SUPRARENAL CORTEX. The suprarenal body exerts an effect on carbohydrate metabolism not only through its medulla, which liberates adrenaline into the blood, but also through the cortex. It is well recognised that after removal of the suprarenals the absorption of monosaccharides from the intestine is retarded, and the glycogen stores of the body reduced, while the blood sugar is lowered and the blood lactate raised.

Further study has shown that the effects of removal of the suprarenals closely resemble those of hypophysectomy. Thus there is the same tendency to hypoglycæmia on fasting, the same lowered glycogen reserves, especially on fasting, the same sensitivity towards insulin, and, what is even more important, the same amelioration of diabetic symptoms in the pancreatectomised animal. The diabetogenic action of pituitary extracts, moreover, is reduced by adrenalectomy. This is especially well seen in the partially depancreatised rat, which is very sensitive to anterior pituitary hormones

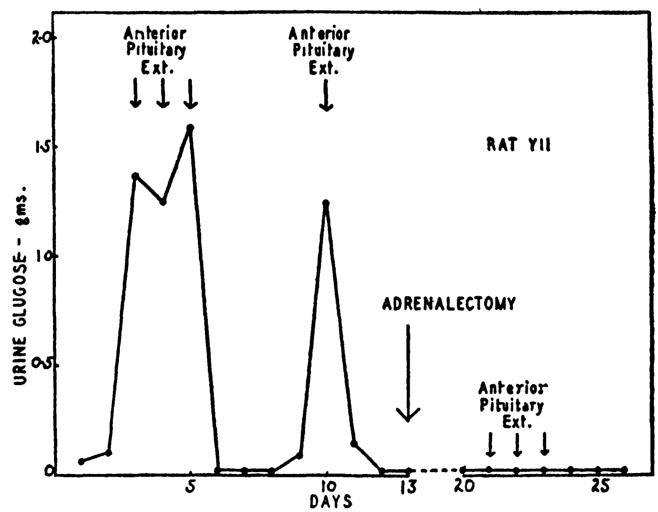

FIG. 580. Effect of adrenalectomy on the glycosuric response to anterior pituitary extract in the partially depancreatised rat. (LONG. Harvey Lectures, 1937.)

(Fig. 580). When extracts of the suprarenal cortex, or the cortical hormones are injected, effects similar to those produced by anterior pituitary extracts ensue. Thus, in fasting hypophysectomised rats there is a rise in the blood sugar and in the store of glycogen, as the following summary shows :—

MEAN CARBOHYDRATE LEVELS OF FASTED RATS INJECTED WITH CORTICAL HORMONE.*

Treatment.	Muscle Glycogen. mg/100g.	Liver Glycogen. mg/100g.	Blood Glucose. mg/100g
Normals, untreated	521	188	84
Hypophysectomised, untreated	320	94	52
Hypophysectomised, given 0·03 mg. Kendall's Comp. B every hour for 24 hours	450	1,520	107

* LONG and KATZIN. *Proc. Soc. exp. Biol.*, 1938, **38**, 516 ; KATZIN and LONG. *Amer. J. Physiol.*, 1938, **123**, 113.

Consideration of these facts has led to the conclusion that many of the effects connected with the pituitary body are actually effected through a stimulating effect which this has, through one of its hormones, on the suprarenal. After hypophysectomy, the suprarenal lacks this stimulus to produce or liberate its cortical hormones, and the reduction of glyconeogenesis is partly due to this. Not all of the effects are likely to be due to this, however, since anterior pituitary extracts are still diabetogenic, though less so, after adrenalectomy. It is probably expedient, therefore, to assume that the pituitary hormones affect carbohydrate metabolism in two ways; first by their stimulating effect on the suprarenal, and secondly by a direct effect in stimulating glyconeogenesis in the liver.

REGULATION OF THE BLOOD SUGAR. We thus see that the regulation of the blood sugar involves a balance of several competing factors. Apart from the entry of carbohydrate into the body during absorption from the intestine, glycogen is constantly being produced in the liver, certainly from protein, probably also from fat, by processes of glyconeogenesis. According to the extent to which the carbohydrate requirements of the body are being met by absorption from the intestine, this glycogen is converted into glucose and liberated, rapidly in fasting, slowly during the well-fed state, into the blood passing the liver. The process of glyconeogenesis is accelerated by the diabetogenic hormone of the pituitary, partly through its action on the suprarenal, and is checked by insulin. Conversion of glycogen in the liver into glucose is accelerated by adrenaline. When the blood sugar tends to rise, more insulin is set free from the islets of the pancreas, and this not only checks glyconeogenesis in the liver, but also accelerates glycogen storage in the muscles and oxidation of glucose in the tissues. When the blood sugar tends to fall, adrenaline is liberated into the blood stream, and conversion of glycogen to glucose in the liver and of glycogen into lactic acid in the muscles is speeded up, until the blood sugar level is set right again.

Removal of the pancreas not only depresses the oxidation of glucose in the tissues, but also leaves the process of glyconeogenesis in the liver unchecked. Removal of the liver, or of the pituitary, or, to a lesser extent, the suprarenal, leaves the glucose-removing functions of the pancreas in charge of the situation. Proper regulation of the blood sugar is only got by appropriate collaboration between all these various factors.

Diabetes in Man. There is good reason to suppose that diabetes in man may be due either to excessive secretion of the pituitary "diabetogenic" hormone, or to inadequate secretion of insulin by the pancreas. The latter form seems much the more common. It has been shown by Himsworth * that there are two types of diabetic, one sensitive to insulin and one less, or more slowly, responsive. The sensitive type, if given glucose and insulin, show an almost normal response to the latter; the insensitive type, on the other hand, shows the great rise of blood sugar in response to the glucose, as though no insulin had been given (Fig. 581). It is suggested that the insulin-sensitive types, which are usually the more severe, are due to lack of insulin in the body. while the insensitive types are due to impairment of the action of insulin on the tissues, as happens in normal individuals on low carbohydrate diets, presumably because of over-production of pituitary hormones. In its severer forms, the diabetes of man resembles very closely that produced in the dog by total extirpation of the pancreas. The output of urine is largely increased, and the urine, though light in colour, is of a high

* HIMSWORTH, *B.M.J.*, 1940, *i.*, 719.

specific gravity, 1030 to 1035, and may contain from 5 to 10 per cent. of sugar. The appetite is largely increased, yet the body wastes. The patient after some months or years may die in a condition of diabetic coma. Warning of the onset of this condition is given by the rise of ammonia in the urine and by the appearance of β-hydroxybutyric and aceto-acetic acids. The breath may smell of acetone, and this substance may also be present in the urine. The diabetic state is attended by diminished resistance of the tissues to infection. In a few cases the pancreas is found to be atrophied or diseased, but in the large majority no marked pathological change is to be observed with the naked eye in this organ. Wherever it has been possible to apply refined methods of miscroscopical examination, some change has often been found in the islets of Langerhans (Opie). We may find all grades between such cases and

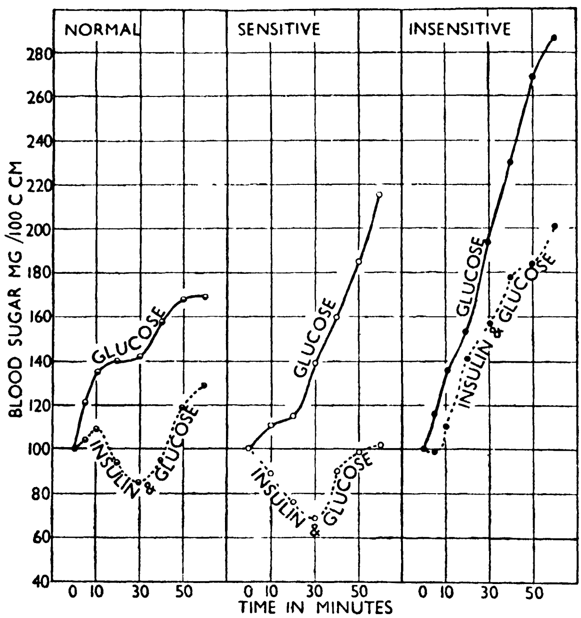

FIG 581 Glucose tolerance and insulin-glucose curves from a normal subject, from an insulin sensitive diabetic and from an insulin-insensitive diabetic, all on same diets (HIMSWORTH and KERR, *Clin Sci*, 1939, **4**, 119)

those in which there is still a considerable power of glucose assimilation. In order to determine the grade of the disorder, it is usual to give a glucose test meal, and to examine the urine for sugar and make a blood-sugar curve. In many cases the sugar will disappear from the urine on the administration of a diet consisting entirely of proteins and fats. When this has been effected, carbohydrates may be added in small proportions to the diet up to the limit of the assimilatory powers of the patient. Some think that administration of carbohydrate in excess of this limit is of disadvantage to the patient and hastens the progress of his disorder. When the power of assimilating carbohydrates is entirely abolished, death is inevitable unless insulin be given. This point may be determined in two ways. In the first place, a patient with no power of carbohydrate assimilation will continue to excrete sugar in the urine on a pure protein-fat diet. Information may also be obtained from a study of his respiratory quotient. A very low respiratory quotient is a sign of severity of the disorder.

As in the diabetes produced by extirpation of the pancreas, the patient may often be restored to health, and kept in that condition, by the repeated administration of hypodermic doses of insulin. Increasing the carbohydrate in the diabetic diet does not necessitate a corresponding increase in the amount of insulin required, because, as with normal individuals, the carbohydrate tolerance and the insulin sensitivity are raised when the carbohydrate in the diet is increased. Care must be taken not to give too large a dose of insulin, as the hypoglycæmia thereby produced is more immediately dangerous than the hyperglycæmia of the diabetic condition. This state of hypoglycæmia, if accidentally induced by an overdose, can be removed by administration of glucose, either orally or by subcutaneous injection.

A drawback to the treatment by insulin is that, unless very frequent small injections be given, the dosage is rather sudden and irregular. Many attempts have been made to find some means of spreading out the action of a given dose of insulin so as more closely to imitate the steady production by the pancreas. The most useful of these are by the use of zinc insulinate and protamine zinc insulinate, both of which are more slowly absorbed and so produce more smooth effects. If the administration of insulin be intermitted, the patient returns at once to the diabetic condition.

Intermediary Metabolism of Carbohydrate. The stages by which carbohydrate is oxidised in the body, with final formation of CO_2 and water, are only partially known They are dealt with in Chapter X.

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BOOK VII

EXCRETION AND TEMPERATURE REGULATION

CHAPTER XLVII

RENAL EXCRETION

1. THE COMPOSITION AND CHARACTERS OF THE URINE

WATER, taken with the food, or derived from the oxidation of hydrogen, is got rid of by the lungs, skin, and kidneys. The salts of heavy metals, *e.g.* bismuth, mercury, when administered, are excreted for the most part by the alimentary canal. The residues of the bile and digestive secretions are also eliminated with the fæces. With these exceptions, practically all the waste products, except CO_2, resulting from metabolism are excreted in the urine by the kidneys. We have thus in this fluid the last chapter in the metabolic history of a large number of the constituents of the diet. Since, moreover, the kidneys may excrete almost any substance which circulates through their blood vessels, many of the intermediate metabolites may be found in minute quantities in the urine. Under pathological conditions, these metabolites may appear in the urine in larger amounts and serve then as an index to some interference with metabolism.

The composition of the urine must, therefore, vary according to the activity of the body, the quantity and nature of the food taken, and the relative amount of water escaping by other channels. But we can describe an average composition for the urine. The history of the urinary constituents has been given for the most part in the chapter dealing with metabolism, but we will enumerate in this chapter the various constituents of the urine and summarise their properties and normal significance.

The urine of man is a clear yellow fluid, which froths when shaken. On standing, a cloud of mucus is deposited, consisting of a very small amount of nucleoprotein derived from the epithelial lining of the bladder and urinary passages. In concentrated urine a deposit occurs on cooling. This deposit dissolves when the urine is warmed, and consists of *urates*. When normal urine is turbid as it is passed, the turbidity generally consists of earthy phosphates and is not cleared up by heating, but dissolves in dilute acid.

The *colour* of the urine varies with its concentration. After severe sweating the amount of water excreted by the kidneys is small, and the urine is therefore of high colour. After copious draughts of liquid the urine may be very pale.

Ordinary urine has an aromatic *odour*, but this varies largely with the character of the food.

The *specific gravity* of the urine is proportional to its concentration. Normally it is 1016 to 1020, though it may rise as high as 1040 or sink as low as 1002.

Its osmotic pressure is usually much above that of the blood plasma, Thus, the Δ of urine normally varies between 0·87 and 2·71° C. (Δ of blood = 0·56°). After copious draughts of water the depression of freezing-point of the urine may be as small as 0·25° C.

The *reaction* of urine is generally acid. This is due to the fact that neutral constituents of the food may give rise to acid end-products of metabolism. The sulphur of proteins is converted into sulphuric acid and the phosphorus of nucleic acid and lecithin into phosphoric acid. There is thus a predominance of acid radicals over bases in ordinary urine. The pH of the urine can easily be determined by indicators, is on the average 5·3, and varies from 7·5 to 5·0.* This, however, applies only to man and to carnivora. In the food of herbivora there is a predominance of alkaline bases. Salts of vegetable acids, *e.g.* tartaric, malic, and citric acids, undergo oxidation in the body, so that their bases remain behind and the urine of herbivora therefore is alkaline in reaction, and turbid. If a herbivorous animal be starved, so that it has to live on its own tissues, its urine becomes clear and acid. The urine of man can be made alkaline by the ingestion of large quantities of vegetables, by the administration of alkalies (*e.g.* $NaHCO_3$) by the mouth, or by prolonged forced breathing.† Under such circumstances, the urine, as passed, is generally turbid from the presence of precipitated earthy phosphates.

In determining the titratable acid of the urine, it is usual to adhere to one indicator, *e.g.* phenol red, and to give the acidity in terms of decinormal acid, naming the indicator used. The total acid eliminated per day is equal to about 700 c.c. of N/10 acid, but some is excreted as ammonium salts ($-\bar{c}$ 400 c.c. N/10 acid daily). The rest, the titratable acid, amounts to about 300 c c $\frac{N}{10}$ acid daily.

Owing to the buffering action of phosphates, the *titratable acidity* of urine is always much greater than the H-ion concentration would suggest. By titrating, first with methyl orange and an acid, and then with phenolphthalein and an alkali, we obtain a useful measure of the buffers of the urine. By the first titration we convert the dibasic phosphates to the monobasic salt (NaH_2PO_4), and by the second we convert the monobasic to the dibasic phosphate (Na_2HPO_4). Hence the ratio of the first to the second titration gives us the ratio $\frac{Na_2HPO_4.}{NaH_2PO_4.}$ (Leathes. ‡)

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THE AVERAGE COMPOSITION OF THE URINE. The following may be taken as a fair daily average for an adult man on ordinary mixed diet, in Europe:

Total amount of urine = 1500 c.c.

This contains about 60 g. of solids, of which 25 g. are inorganic and 35 g. organic. They are distributed as follows:

Inorganic Constituents		Organic Constituents	
Sodium chloride	15 0 g	Urea	30·0 g.
Potassium	3 3 ,,	Creatinine	1·0 ,,
Sulphuric acid	2 5 ,,	Uric acid	0·7 ,,
Phosphoric acid	2·5 ,,	Hippuric acid	0·7 ,,
Ammonia	0·7 ,,	Indican	0·01 ,,
Magnesium	0·1 ,,	Acetone bodies	0·04 ,,
Calcium	0·3 ,,		
Other substances	0·2 ,,		
Iron	0·005 ,,		

* HENDERSON and PALMER. *J. Biol. Chem.*, 1912, **13**, 394.
† DAVIES, J. B. S. HALDANE and KENNAWAY. *J. Physiol.*, 1921, **54**, 32.
‡ LEATHES, *B. M. J.*, 1919, p. 165; *Lancet*, 1920, ii., 933.

of blood pressure, but intense pallor due to cutaneous vaso-constriction. The cardiac output may be diminished owing to constriction of the coronary vessels. According to Krogh, a certain content of pituitrin in the circulating blood is essential for the maintenance of the normal tone of the capillaries in the frog.

(2) Contraction of plain muscle generally. Administration of pituitary extract to a lactating animal leads to sudden expulsion of milk, due to contraction of the plain muscle in the walls of the milk sinuses. There is also contraction of the plain muscle of the alimentary canal. The production of contraction of plain muscle of the uterus is called the *oxytocic* action.

(3) Expansion of the melanophores (pigment cells) of the frog's skin (Hogben and Winton), so that the skin looks darker in colour. (Especially by extracts of the pars intermedia.)

(4) *Anti-diuretic Effect.* In anæsthetised animals, a brief diuresis follows the injection. This is due to the rise of blood pressure. If injected into a normal man who has received a large draught of water (1 l.) pituitrin delays for four or five hours the excretion of the water by the kidneys, so that it accumulates for a time in the tissues and may even cause an actual dilution of the blood. Ultimately a pathological condition, water poisoning, may develop owing to excessive retention of water. The effect appears to be due to an acceleration of water reabsorption in the kidney tubules. It also increases the excretion of certain ions, *e g.*, Cl, as has been shown with the heart-lung-kidney preparation (p 1064). There may be a temporary increase in the urine, with diminution of chlorides. The principle causing this water retention is much more abundant in mammalian than in other pituitaries * The condition of diabetes insipidus, however brought about, may be relieved temporarily by the subcutaneous injection of a watery extract of the posterior lobe.

(5) Hyperglycæmia, glycosuria, lowered sugar tolerance, loss of liver glycogen, and an action antagonising that of insulin.

There is also a rise in the fat of the liver, and a decrease in basal metabolic rate. To what extent these effects are due to a specific hormone or to hormones from the anterior lobe is uncertain.

The best known actions are the pressor effect, the anti-diuretic effect, and the 'oxytocic' action on uterine muscle. For all these purposes pituitrin, in subcutaneous injection, is employed clinically.

It had long been disputed whether these actions are to be attributed to a single active principle, or whether more than one such hormone is concerned, as was claimed by Dudley. Kamm and others † settled the discussion in favour of the latter, and the separated oxytocic and pressor principles ("*pitocin*" and "*pitressin*") can now be obtained on the market. The cardiovascular and antiduretic effects, and the contraction of most of the plain muscle of the body are due to the pitressin fraction. The contraction of the uterus is a specific effect of the oxytocin, but the human uterus is not always equally sensitive to its action; in the early stages of pregnancy it is ineffective, but as pregnancy advances the sensitiveness increases and reaches a maximum at parturition; after delivery the uterus is again insensitive for some time.

The melanophore expanding principle is a separate substance, derived from the pars intermedia.

The unit of pressor activity is that of 0·1 c.c. of pituitary (posterior

* HELLER. *J Physiol.*, 1941, **99**, 246.

† KAMM *et al.* *J. Amer. Chem. Soc.*, 1928, **50**, 573.

acidity of the urine, the proportion of the two bases present in these forms is diminished. The di-magnesium and di-calcium phosphates are only slightly soluble in water, and would be deposited. One may indeed, in slightly acid urine, find the di-calcium phosphate occasionally present as a crystalline deposit ("stellar phosphate"). On heating the urine, the di-calcium phosphate breaks up into a mono-calcium phosphate and a tri-calcium phosphate, while the acidity of the urine is increased by the solution of the mono-calcium phosphate. Alkaline urine will always present a precipitate of tri-calcium phosphate, $Ca_3(PO_4)_2$. When normal urine is allowed to putrefy, the urea is converted into ammonium carbonate, and the urine becomes alkaline. Under such conditions we may often find a crystalline precipitate of ammonium magnesium phosphate, NH_4MgPO_4, the so-called 'triple phosphate.'

(*b*) THE BASES OF THE URINE. The bases include potassium, sodium, ammonium, magnesium and calcium.

The amount of *potassium* excreted per day varies between 2 and 3 g., according to the food. With a large meat diet, and in fasting, the output is increased.

The amount of *sodium* excreted in the twenty-four hours is on the average about 5 g., but fluctuates largely with the diet.

Calcium and *magnesium* are invariably present in urine, but in much smaller quantities than the alkaline metals. The average amount of these two bases in the twenty-four hours varies in each case between 0·1 and 0·2 gr.

Normal human urine always contains a small amount of *ammonia*, on an average between 0·6 and 0·8 g. in the twenty-four hours. As we have already seen, in dealing with the origin of urea, the quantity of ammonia in the urine is an index to the excess of acids over bases which have to be excreted. According to Nash and Benedict,* the ammonia of the urine is almost entirely formed in the kidney itself, from amino acids or urea, and does not represent an excretion from the blood, from which it is absent. The experiments of Bliss,† however, throw some doubt on this, and suggest that ammonia can be, and is, formed in all the tissues, including the kidney.

Traces of *iron* may be excreted by the urine, as a rule less than 5 mg. per day.

ORGANIC CONSTITUENTS OF THE URINE

Several of these constituents contain nitrogen, which in man on an ordinary mixed diet is distributed among the various urinary constituents as follows:

Urea	85–90 per cent.
Ammonia	2–4 ,,
Creatinine	3 ,,
Uric acid	1–3 ,,

About 6 per cent. of the urinary nitrogen is in the form of other substances such as hippuric acid, pigments, &c. All the organic constituents of the urine (except hippuric acid) are obtained direct from the blood; hence they represent real excretory products. Their origin in the body has already been discussed in Chapter XLVI.

Urea, $CO\langle{NH_2 \atop NH_2}$ or $NH:C\langle{NH_3 \atop O}$ ‡. From 20 to 35 g. per day are

* NASH and BENEDICT. *J. Biol. Chem.*, 1921, **48**, 463; BENEDICT and NASH, *Ibid.*, 1929, **82**, 673.

† BLISS. *J. Biol. Chem.*, 1926, **67**, 326.

‡ WERNER. Urea. (Longmans, Green.) 1923.

usually excreted. Urea may easily be prepared from urine by evaporating to dryness with animal charcoal and extracting the dry residue with acetone. On evaporation of the filtered extract, urea crystallises out.

Urea is hydrolysed by certain micro-organisms, *e.g.* the *micrococcus ureæ*, which is responsible for the ammoniacal change which occurs in urine when exposed to the air: $CON_2H_4 + H_2O = CO_2 + 2NH_3$.

This action is due to an enzyme, *urease*, which is also present in certain plant tissues, *e.g.* the seeds of the soya bean. When urea, in slightly acid solution, is incubated with ground soya bean, the urea is all converted into ammonium salts; on making the fluid alkaline the ammonia is liberated and may be removed by a current of air and absorbed in a standard acid. This forms the basis of a method for the estimation of urea.*

Urea solutions when mixed with alkaline hypobromite solutions evolve nitrogen :—

$$CON_2H_4 + 3NaOBr = CO_2 + N_2 + 2H_2O + 3NaBr.$$

The CO_2 formed is absorbed by the excess of caustic alkali, and if the evolved nitrogen be measured an approximate estimate of the urea is obtained.

Another test and method for estimation of urea is based on the fact that when a solution of urea, acidified with acetic acid, is mixed with a solution of xanthydrol $\left(C_6H_4\left\langle\begin{matrix}CHOH\\ —O—\end{matrix}\right\rangle C_6H_4\right)$ in methyl alcohol, a white insoluble precipitate is formed, which contains exactly one-seventh of its weight of urea.†

Urea, being the chief nitrogenous constituent of the urine, is the most important index to the protein metabolism of the body. It may be regarded as partly exogenous, partly endogenous. The greater part excreted by a normal individual in the course of the day is exogenous, and we have already seen that it is formed in the liver (Chapter XLVI.).

Creatinine. Creatinine is a normal constituent of urine, in which it occurs in quantities of 1·0 to 1·5 g. in the twenty-four hours It can be prepared from urine by precipitation as picrate, and conversion into the zinc chloride compound, which is then decomposed by ammonia ‡

On treating a solution of creatinine with a few drops of a watery solution of picric acid and dilute caustic potash, an intense red colour is produced. This reaction is made use of for the quantitative estimation of creatinine.§

Uric Acid. 2-6-8-trioxypurine. The amount of uric acid per day varies, according to the diet, from 0·1 to 2·0 g. Uric acid can be prepared from urine by precipitation, as ammonium urate, with excess of ammonium chloride and ammonia. The precipitate is collected and treated with dilute acid, when uric acid crystallises out, or can be estimated quantitatively by titration with permanganate.‖ More usually uric acid is estimated colorimetrically by treatment with phosphotungstic acid, with which it gives a blue colour in alkaline solution.¶

It is insoluble in water, but soluble in concentrated acids and in alkalies.

* Van Slyke and Cullen. *J. Biol. Chem.*, 1914, **19**, 141; Youngburg. *J. Biol. Chem.*, 1921, **45**, 391.

† Fosse. L'Urée. 1928. (Press Univ. de France.)

‡ Benedict. *J. Biol. Chem.*, 1914, **18**, 182.

§ Folin. *J. Biol. Chem.*, 1914, **17**, 469.

‖ Folin. *Z. f. physiol. Chem.*, 1898, **24**, 224.

¶ Folin and Wu. *J. Biol. Chem.*, 1919, **38**, 459; Benedict. *Ibid.*, 1922, **54**, 233.

Uric acid acts as a weak dibasic acid. It forms three orders of salts, namely, the neutral urates, the bi-urates and the quadri-urates. The neutral urates, $Na_2\bar{U}$, are highly hydrolysed and give strongly alkaline solutions. The bi-urates, $NaH\bar{U}$, are the most stable of the urates. They may be prepared by dissolving uric acid with the aid of heat in weak solutions of the alkaline carbonates, from which they separate, on cooling, in stellar crystals. The quadri-urates have the formula $H_2\bar{U}$, $NaH\bar{U}$. It is probable that the greater part of the uric acid in the urine and the so-called 'lateritious deposit,' the brick-red amorphous precipitate which occurs in concentrated urine on cooling, consists of these quadri-urates. The exact condition of the urate, however, will depend on the reaction of the urine. When the urine is acid, there will be a tendency to the precipitation of uric acid.

Small traces (16 to 60 mg. per day) of *purine bases*, namely, xanthine, hypoxanthine and adenine, also occur in urine. When tea and coffee are taken the methyl purines may occur, namely, caffeine, theobromine and their derivatives.

Allantoin is only present in traces in human urine (5 to 15 mg. per day).

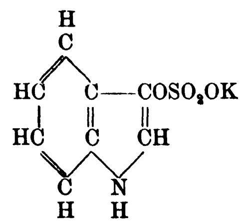
Amino-Acids. Amino-acids are always present in traces in the urine, and contribute per day from 0·4 to 1·0 g. of nitrogen.

Hippuric Acid or benzoyl glycine, $C_6H_5CO{\cdot}NH{\cdot}CH_2COOH$, is a frequent, though not a constant, constituent of human urine. It is derived from benzoic acid, or from substances which, on oxidation, can give rise to benzoic acid.

In the dog this coupling can be effected in the kidneys, but in most other animals, including man, it is probable that the synthesis occurs mainly in the liver. In fact, in diseases of the liver, administration of benzoic acid is followed by a slower excretion of hippuric acid than is seen in normal subjects, and this has been made the basis of a liver-function test.*

The amount of hippuric acid excreted in the day may vary between 0·1 and 1 g. After a diet rich in fruit or vegetables its amount may rise to 2 g. It is present in considerable quantities in the urine of herbivora and may be most easily prepared from horses' urine. It forms milk-white crystals, which are only slightly soluble in cold water, but easily soluble in alcohol and in ethyl acetate.

Other Aromatic Substances. The chief of these (about 5 to 20 mg. per day) is the so-called 'urinary indican' or potassium-indoxyl-sulphate. This is derived from the indol produced in the intestines from the tryptophan in the proteins of the food, the change being effected by putrefaction. Urinary indican has the formula :



It is converted into indigo by the action of mild oxidising agents (*e.g.* HCl and ferric chloride), and when subsequently shaken with chloroform the chloroform layer is seen to be deep blue.

* Quick. *Arch. Int. Med.*, 1936, 57, 544.

The Urinary Pigments. Normal urine gives no definite absorption bands. It owes its colour to the presence of a yellow pigment, *urochrome*. In order to separate urochrome, urine is acidified slightly with acetic acid and extracted with $\frac{1}{10}$ its volume of *n*-butyl alcohol. After washing and concentrating the alcoholic solution, it is again washed with chloroform, benzene and ether; then after further concentration the solid urochrome crystallises out as a brown powder.* The pigment is precipitated by various salts of heavy metals, and is decolourised by reduction with zinc and HCl. About 73 mg. of urochrome is excreted per day by an average adult, the amount varying with the basal metabolism. Urochrome, on treatment with aldehyde, yields a pigment closely similar to urobilin. On the other hand, urobilin, evaporated with wet ether, is converted into urochrome. Urochrome is therefore probably derived from urobilin, possibly by union with a polypeptide.

Urobilin is only present in traces in normal urine, and then chiefly in the form of a chromogen, *urobilinogen*, from which it must be set free by acidification. In certain pathological conditions, especially in cirrhosis of the liver, urobilin may occur in the urine in considerable quantities.

The urobilin of urine, which has now been obtained in pure crystalline form, is closely related to stercobilin, the colouring matter of the fæces.† It is formed in the intestines by the reducing action of micro-organisms on bilirubin, and urobilinogen is a result of further reduction.

Urobilin may be detected in the urine by Schlesinger's test. The urine is acidified with acetic acid and extracted with amyl alcohol. The amyl alcohol layer is removed and a few drops of alcoholic zinc chloride added, when a green fluorescence appears.

Urobilinogen is detected by Ehrlich's test—addition of a solution of *p*-dimethylaminoazobenzaldehyde in 50 per cent. HCl gives a red colour.

Other pigments which may occur in urine are uroerythrin and porphyrins. *Uroerythrin* gives the pink colour to urate sediments. Its chemical nature is not known. It is distinguished by the fact that on addition of caustic soda the pink colour is changed to green. On suspending the red-coloured precipitate of urates in hot water and extracting with amyl alcohol, a pink solution is obtained which shows two absorption bands in the green part of the spectrum.

Porphyrins of series I (coproporphyrin I, and uroporphyrin I) are present only in very small amounts in normal urine, but under certain conditions, especially after poisoning with sulphonal, may occur in such large quantities as to give the urine a deep purple colour. Under these circumstances they are found in the form of alkaline porphyrins and give the characteristic absorption bands of the latter.

NON-NITROGENOUS ORGANIC CONSTITUENTS. The chief of these are *oxalic acid*, of which 15 to 20 mg. per day are excreted, though more is present if oxalates are taken by mouth; and *lactic acid*, the amount of which, usually 75 to 200 mg. per day, varies with the amount of muscular exercise recently taken. Traces of aceto-acetic and β hydroxybutyric acid are also present.

ABNORMAL CONSTITUENTS OF THE URINE‡

A large number of substances occur in minute traces in the urine. Most of the so-called pathological constituents may be detected in traces in normal urine. It is only when they occur in easily detectable amounts that their presence becomes of significance.

* DRABKIN. *J. Biol. Chem.*, 1930, **88**, 433, 443.
† WATSON. *Ztschr. f. physiol. Chem.*, 1935, **233**, 39.
‡ For further details *v.* HARRISON. Chemical Methods in Clinical Medicine. 1930. (Churchill.)

Coagulable Protein. Under normal circumstances, urine is free from protein except for the small traces of mucinous material which gives a cloudy deposit. When foreign proteins of a molecular weight lower than about 68,000 are present in the blood, these are excreted in the urine; proteins of higher molecular weight, though foreign to the blood stream, are not passed out by the normal kidney.* If the kidney cells are damaged by disease, by interference with their blood supply or by circulating poisons, the glomerular epithelium permits the passage of a certain amount of the serum albumin and serum globulin of the plasma, and on histological examination the coagulated protein may be seen in Bowman's capsule. The presence of coagulable protein (generally spoken of as albumin) in the urine is associated with Bright's disease. A small trace will often be found in the urine which is passed shortly after taking muscular exercise, but this has no pathognomonic significance.†

Hæmoglobin or its derivatives is present in the urine when hæmolysis occurs in the blood stream or urinary passages.

Sugar. In diabetes, glucose appears in large quantities in the urine. In renal glycosuria glucose appears in the urine, but the blood sugar is rather lower than normal. Lactose may occur in the urine of nursing women.

In rare circumstances, fructose or a pentose may be found in the urine.

Glucuronic Acid. About 150 mg. are excreted daily in normal conditions. Most of it is conjugated with other substances to form glucuronides, and this conjugation, effected in the liver, is one means by which poisonous substances when administered or when found in the body are detoxicated. Camphor, chloral, and to some extent phenol, indol and skatol, are excreted as glucuronides. Certain steroids related to the sex hormones, *e.g.* pregnandiol, are also excreted in the form of glucuronides. Glucuronic acid is an oxidation product of glucose, having the formula:

$$\begin{array}{c} COOH \\ | \\ (CHOH)_4 \\ | \\ CHO \end{array}$$

It reduces Fehling's solution and is lævorotatory.

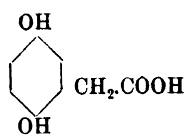
Acetone Bodies. These substances are present in traces (20 to 50 mg. per day) in normal urine, and in large amount in diabetes, especially towards the end of the disease. They represent the penultimate stages in the oxidation of the fats. Their relation to one another is seen from their formulæ:

$$\begin{array}{ccc} CH_3 & CH_3 & CH_3 \\ | & | & | \\ CHOH & CO & CO \\ | & | & | \\ CH_2 & CH_2 & CH_3 \\ | & | & \\ COOH & COOH & \\ \beta\text{-Hydroxybutyric acid} & \text{Aceto-acetic acid} & \text{Acetone} \end{array}$$

They may also occur in any condition of carbohydrate starvation, relative or absolute.

Cystine. This is found in the urine of certain individuals.‡ The condition of cystinuria represents, like alcaptonuria, an inborn error of metabolism, and persists throughout life. In such cases, the cystine may give rise to urinary deposits or even to a urinary calculus.

Homogentisic Acid. This is dihydroxyphenyl acetic acid. Its formula is as follows:



* KERRIDGE and BAYLISS, *Lancet*, 1932, p. 785; KERRIDGE, RUSSELL and BAYLISS, *J. Physiol.*, 1933, **77**, 386.

† MACLEAN. Renal Disease. (London.) 1927.

‡ ROSENFELD. *Ergebn. d. Physiol.*, 1920, **18**, 118.

It occurs in a quantity of 3 to 6 g. per day in the urine of individuals affected with *alcaptonuria*. It slowly darkens on exposure to the air, and on the addition of alkali and shaking with air it becomes black. It reduces Fehling's solution, so that the presence of sugar may be suspected. Its origin has already been dealt with.

Bile Pigments. Bilirubin and biliverdin are present in the urine in jaundice, owing to the presence of bilirubin in greatly increased amount in the blood plasma.

Bile Salts are also often detectable in the urine in obstructive jaundice, *e.g.* in jaundice due to gall-stones.

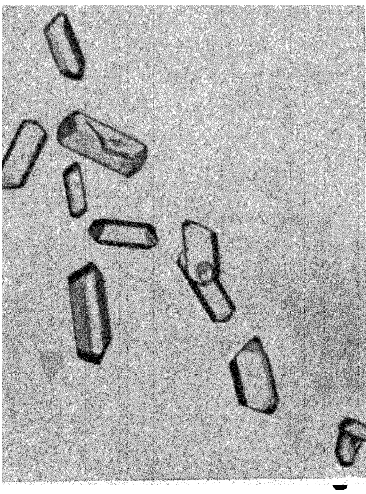


FIG. 582. Triple phosphate (knife-rest form). × 145

FIG. 583. Triple phosphate (feathery form). × 145.

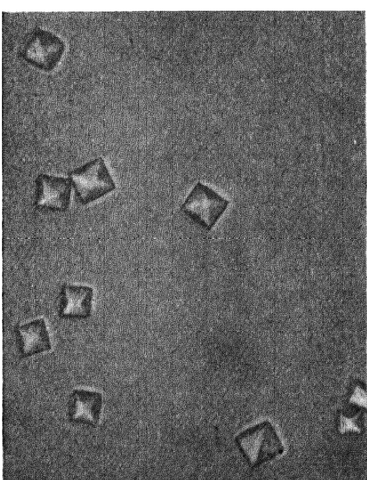


FIG. 584. Calcium oxalate (envelopes). × 640.

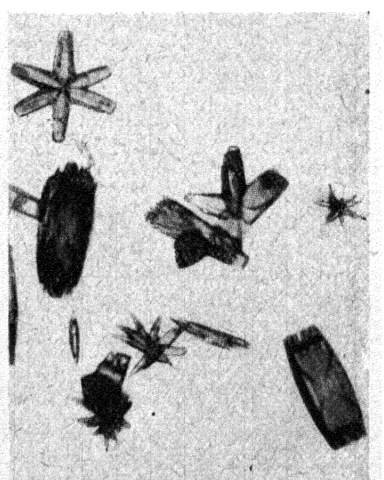


FIG. 585. Uric acid (barrels, stars, etc.). × 66.

FIGS. 582–585. Urinary deposits.

(From THORPE's 'Biochemistry for Medical Students.' (Churchill.))

Urinary Deposits. In addition to formed elements, such as blood corpuscles, bacteria, or pus cells, which may occur in abnormal urine, the following deposits may be found :

(*a*) *In Acid Urine.* (1) Amorphous urates occur generally as a brick-red amorphous deposit, thrown down as the urine cools. It is redissolved on warming the urine, and consists generally of the quadri-urates. The acid urate of sodium and of ammonium may occasionally occur in star-shaped clusters of needles or as spherules with small crystals adhering to them.

(2) Uric acid. Whetstone, dumb-bell, or sheaf-like aggregations of crystals, generally deeply pigmented so as to resemble cayenne pepper (Fig. 585).

(3) Calcium oxalate (Fig. 584). Colourless, transparent, highly refractive octahedral crystals (envelope-shaped). Insoluble in acetic acid, soluble in hydrochloric acid.

(4) Ammonium magnesium phosphates (in faintly acid or neutral urine). The crystals have been compared to knife-rests or coffin-lids (Fig. 582). They are soluble in acetic acid.

(5) Calcium hydrogen phosphate, $CaHPO_4$. They form prismatic crystals often arranged in rosettes. Easily soluble in dilute acetic acid. On adding a solution of ammonium carbonate, the crystals are eaten away and form an amorphous deposit.

(6) Tyrosine, fine needles in star-shaped bundles, and cystine, in regular hexagonal plates, may occur under very rare circumstances.

(*b*) *In Alkaline Urine.* (1) The commonest precipitate consists of earthy phosphates, amorphous, easily soluble in dilute acetic acid.

(2) Ammonium magnesium phosphate, or triple phosphate (Fig. 583), is common in urine which has undergone ammoniacal fermentation.

(3) Acid ammonium urate may also occur in alkaline urine. On treatment with HCl it is dissolved and uric acid in crystals slowly separates out.

2. THE SECRETION OF URINE

With the exception of hippuric acid in the dog, and perhaps ammonia, all the constituents of the urine are formed in parts of the body other than the kidneys, which merely excrete them from the blood. Hence extirpation of both kidneys leads to an accumulation of these specific urinary constituents in the blood and tissues. Considered from a broad standpoint, the function of this organ is the preservation of the normal composition of the circulating blood. Whenever the latter contains an abnormal non-colloidal constituent, or normal constituents in abnormal quantities, the kidney excretes the substance in question until the normal composition of the blood is restored. The problem is to determine the conditions which influence the quantity and quality of the urine secreted by the kidneys, and to ascribe to each structural element its proper share in the total work of the kidney.

Structure of the Kidney. Our views as to function are intimately dependent on our knowledge of the structure of the kidney. This organ is a branched tubular gland consisting, in man, of ten to fifteen nearly equal divisions, known as the Malpighian pyramids. In certain animals, such as the rabbit and rat, only one pyramid is present. It is divided into an outer portion, or cortex, an inner portion, the medulla, and between these the 'boundary layer,' containing the larger branches of the renal blood-vessels (Fig. 586). From the outer boundary of the Malpighian pyramids of the medulla, a number of processes, the medullary rays, pass out into the cortex towards the surface of the kidney.

The kidney is made up of separate units, or *nephrons*, all of similar structure and function. They are intricate tubules embedded in scanty connective tissue and richly supplied with blood vessels (Fig. 589). Each nephron begins by a blind dilated extremity in the cortex, known as *Bowman's capsule*, which surrounds a bunch of capillary blood vessels, the *glomerulus*, the two together forming the *Malpighian body*. In most mammals there are roughly 2 million glomeruli in the two kidneys for each

sq. m. of body surface.* (In man there may be more.) This means that the number is correlated with the general metabolism of the body. From Bowman's capsule a short *neck* (or 1st segment) leads into a *proximal convoluted tubule* (or 2nd segment), and this into a U-shaped portion which passes down into the medulla, and consists of straight *descending* (3rd segment) *and ascending limbs and the loop of Henle* (Fig. 589). The ascending limb passes into a *distal convoluted tubule* (which, together with the ascending limb of Henle, is called the 4th segment), and this by a *junctional tubule*

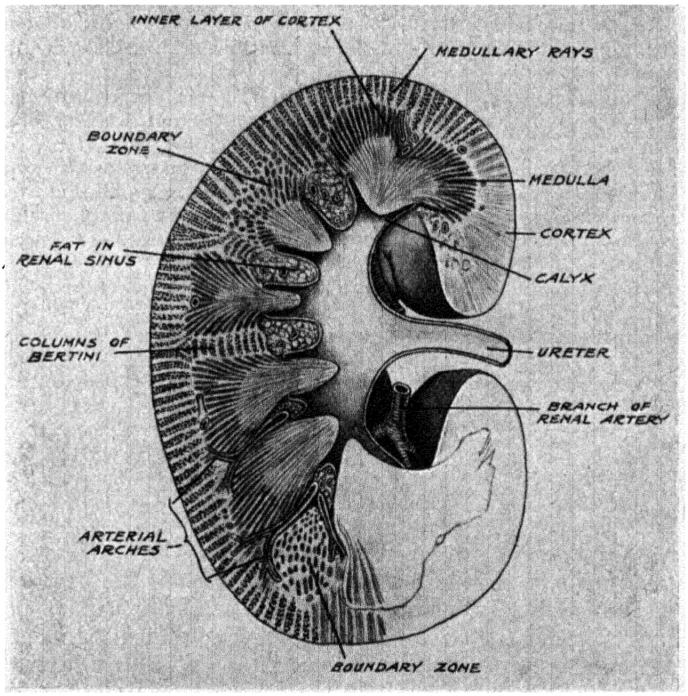


FIG. 586. Longitudinal Section of the Kidney, opening up the Kidney Sinus. The pelvis of the ureter and some of its calyces have been laid open as they lie within the sinus. (RAUBER-KOPSCH.)

(5th segment) joins with a number of others to form a straight *collecting tubule*. Several of these unite to form the papillary ducts, which open on the surface of the papilla in the calyx. The whole tubule consists of epithelium lying on a basement membrane; the epithelium varies in structure in different parts of the tubule. The glomerular capillaries are covered with a very thin layer of endothelial cells, and a similar layer forms the lining of Bowman's capsule. The convoluted tubules contain cells which are cylindrical in cross-section and have long been distinguished as 'rodded epithelium' (Fig. 588) on account of the ease with which a radial disposition of rods or granules is demonstrated in their protoplasm. Moreover, the free margin of the cells, instead of being irregular, consists of a well-marked striated border, formed of a number of very fine hairs closely set together and springing from a row of granules in the peripheral part of the cell (Fig. 588). The hairs, which make up the striated border, have not been observed to present ciliary movement, and are probably comparable with the similar structures found clothing the free border of the epithelium of the intestinal villus. Such cells are characteristic features of the epithelium lining the urinary organs in all types of animals, and are well marked in the nephridia of worms. The descending limb of Henle's loop is narrow, and possesses flattened epithelial cells, while the ascending

* KUNKEL. *Bull. Johns Hopk. Hosp.*, 1930, **47**, 285.

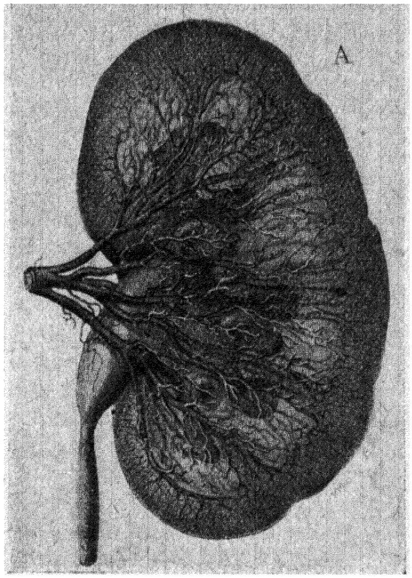


FIG. 587. The Renal Artery and the Distribution of its Branches.

Anterior view of a left kidney. There are six main branches seen entering the kidney substance. Only one of these (the third) passes posterior to the pelvis at the hilum, also small arteries coming from the upper and lower calices. (After MAX BRODEL, *The Johns Hopkins Hospital Bulletin*, Jan., 1901.)

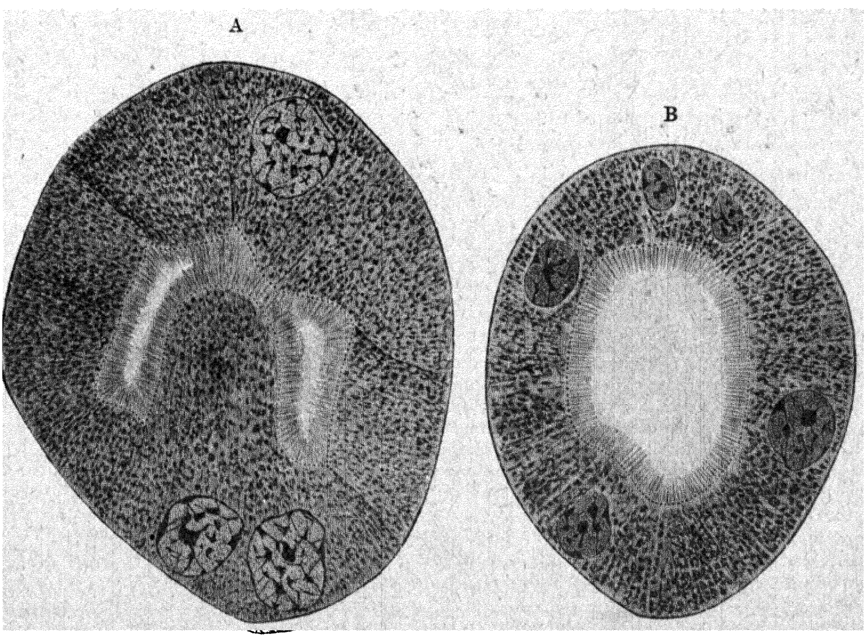


FIG. 588. Cross sections of Convoluted Tubules from Kidney of Rat. (SAUER.)
A. During slight secretion. B. During maximal secretion.

limb presents an epithelium similar to that of the convoluted tubules, but with less marked striation. The junctional and collecting tubules are lined with cubical or columnar cells with a clear protoplasm.

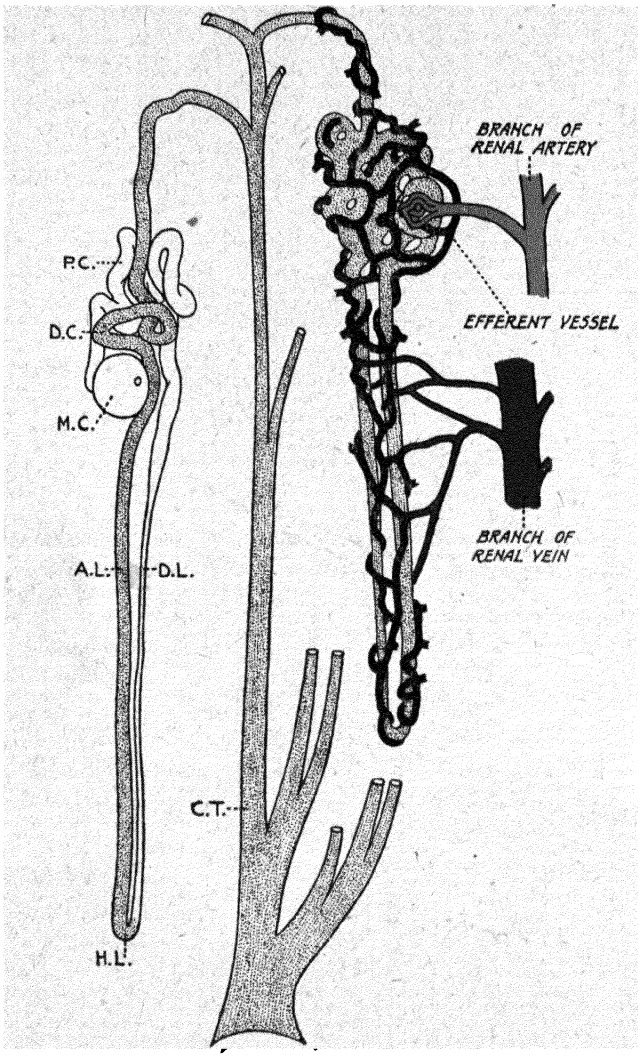


FIG. 589. Diagram of Two Nephrons. On the left, the tubule is outlined from the capsule to the loop of Henle and is shaded from that point to the end of the collecting tubule. On the right, a diagram of the circulation is added. M.C., Malpighian capsule. P.C., Proximal convoluted tubule. D.L., Descending limb. H.L., Loop of Henle. A.L., Ascending limb. D.C., Distal convoluted tubule. C.T., Collecting tubule. (After CUSHNY.)

The clear differences between the structure of these various parts point to a differentiation of function. This conclusion is borne out by a study of the blood supply of the kidney. The renal artery divides in the pelvis into four or five branches (Fig. 587), which pass up to the boundary zone and there give off arteries in different directions; those which run towards the surface are called the interlobular arteries. Each of these, which is an end artery presenting no anastomoses with its fellows, gives off on all sides short wide branches, which pass to the glomeruli and

constitute the *vasa afferentia* of these bodies. Each vas afferens has a thick muscular wall. The glomerulus itself consists of a number of wide capillaries invested by an extremely thin wall. The glomerular capillaries are collected together to form an efferent vessel, the *vas efferens*, which is narrower than the vas afferens and, like the latter, presents a well-marked muscular coat. The vas efferens breaks up again into a second set of capillaries, which ramify round the tubules of the cortex, bunches of long capillaries also passing along the medullary rays and supplying the tubules of the medulla. From the capillaries of the tubules, the blood is collected again into veins, which leave the kidney partly by the cortex and capsular vessels, chiefly by large venous trunks which join to form the renal vein at the hilum of the kidney. The kidney is richly supplied with nerves derived mainly from the 6th to 13th dorsal spinal roots,* which are chiefly distributed to the muscular walls of its blood vessels. Some authors have described a fine nerve-plexus surrounding the tubules and sending branches between and into the cells of the convoluted tubules themselves.

Relation of Structure to Function. The main points in the structure of the kidney was made out by Bowman † and suggested the two theories of urinary secretion, those of Bowman and of Ludwig (1844),‡ which have furnished the basis of all our subsequent investigation of the subject. Both observers appreciated the great difference between the "membrane" covering the glomerular loop and that of the lining of the tubule, and both drew attention to the difference in the circulation in these two portions of the kidney. The glomerular capillaries, supplied with blood through a short wide artery, and drained by an efferent vessel smaller than the afferent, would represent a region of relatively high capillary blood pressure, whereas the pressure in the capillaries surrounding the tubules must be at least as low as that in most other capillary regions. Bowman therefore suggested that the urine arises from two sources, namely, one part containing the water and salts, produced by a process of filtration through the walls of the glomerular capillaries, and another part containing the specific urinary constituents, urea, uric acid, &c. secreted by the cells of the convoluted tubules and added to the dilute glomerular product as it passed on its course along them.

To Ludwig, on the other hand, it seemed possible, at first, to account for the whole process of formation of urine on the assumption that absorption, and not secretion, was the main function of the tubules. He imagined that the whole of the urinary constituents passed through the walls of the vessels of the glomerulus by filtration from the blood. The glomerular transudate, filtering, through what is nowadays called the 'glomerular membrane,' into the Bowman's capsule, would represent, therefore, an ultrafiltrate of blood plasma, and this view still holds the field as the most likely explanation of the functions of the Malpighian body. The great difference in urea content between the blood and the urine Ludwig ascribed to a concentration taking place in the fluid in its passage along the tubules, in which water and certain of the salts were reabsorbed; a process of reabsorption conditioned by the difference in protein content between the urine within the tubules and the tissue fluid and blood under low pressure on the outside of the tubules.

In its original form, the theory of Ludwig is untenable, because the difference of osmotic pressure between blood plasma and its ultrafiltrate is too small to attract from the filtrate back again into the blood enough water to produce the requisite concentration, even of urea, found in urine. For the production of urine from the glomerular filtrate, a certain amount of work would have to be done, by the cells of the tubules, in abstracting the necessary amount of water and returning it to the blood.

* BRADFORD. *J. Physiol.*, 1889, **10**, 358.
† BOWMAN. *Phil. Trans.*, 1842, 57.
‡ LUDWIG. Lehrbuch d. Physiol. des Menschen, 1861, **2**, 373.

The minimum work necessary to form a given amount of urine of a given concentration, from blood of average composition, can be found very roughly by comparison of their osmotic pressures, by any of the methods described in Chapter V. For example, we may find their freezing points. A depression of freezing point of 1° C. in an aqueous solution corresponds to an osmotic pressure of 122·7 m. of water and hence, to concentrate a fluid with a freezing-point of $-1°$ C. to one with a freezing-point of $-2°$ C., would require an expenditure of work equivalent to that required to compress 100 c.c. of a gas at a pressure of 122·7 m. of water to half its bulk. In this way it has been calculated that the work necessary to change a fluid of $\triangle = -0{\cdot}56°$ C. (such as plasma) to one of $\triangle = -2{\cdot}3°$ C. (urine), and to yield 1000 c.c. of the latter would be of the order of 215 kg.m.

More exact calculation would involve the application of thermodynamics, since it would necessitate the separate calculations of the "free energy" changes involved in all the important changes of concentration that occur, in converting the glomerular filtrate into the urine. Such calculations have been made,* and indicate that for average human urine the kidney does work amounting to about 0·7 gram calories per c.c. of urine formed, or 70 gram cals. per gram of nitrogen excreted. This would amount to about 300 kg.m. per litre of urine, or, taking accepted values for the oxygen usages of the kidneys, an 'efficiency' of about 1 per cent. Direct estimation of the constituents of the urine and of the oxygen usages of the kidney also give efficiencies of about 1 per cent. for isolated perfused dog's kidneys.†

The amount of work done in order to bring about concentration is not prohibitively high. In man, the actual work done is really trivial when compared with that effected by the heart, which, though not weighing much more than the two kidneys, would do at least thirty times as much work in the same time.

However, it is certain that the formation of urine of high osmotic pressure from glomerular filtrate of low osmotic pressure does not occur in any simple fashion. If we compare the composition of blood plasma with that of urine, as in the Table given below, we see that the constituents are concentrated to different extents. Moreover, the amount of work to be done, even if it were great, would be no argument against the reabsorption hypothesis, since the same amount of osmotic work must be done, whatever the way by which the

RELATIVE COMPOSITIONS OF BLOOD PLASMA AND NORMAL URINE IN MAN.

	Blood plasma, per cent	Urine, per cent.	Change in concentration in kidney
Water	90–93	95	—
Proteins, fats and other colloids	7–9	—	—
Glucose	0·1	—	—
Urea	0·03	2	60
Uric Acid	0·002	0·05	25
Na	0·32	0·35	1
K	0·02	0·15	7
NH_4	0·0001	0·04	400
Ca	0·008	0·015	2
Mg	0·0025	0·006	2
Cl	0·37	0·6	2
PO_4	0·009	0·27	30
SO_4	0·003	0·18	60
Creatinine	0·001	0·10	100

urine is produced. The large amount of work done, under some conditions, by the kidneys in the formation of urine, is indicated by measurements of the oxygen consumption of this organ. This usually amounts to 2 to 4 c.c. per

* BORSOOK and WINEGARDEN. *Proc. Nat. Acad. Sci.*, 1931, **17**, 3, 13.
† EGGLETON, PAPPENHEIMER and WINTON. *J. Physiol.*, 1940, **97**, 363.

gramme per hour, and in some forms of diuresis may be greater still.* Yet nearly all the energy represented by the oxygen usage is lost as heat, only about 1 per cent. of it appearing as osmotic work.† It is not, therefore, a highly efficient organ from that point of view, but since its work is so important, and so thorough, the energy cost of it is of relatively small moment.

The abandonment of Ludwig's view as to the exact mechanism of the concentration does not, however, place his theory out of court. The questions that will still have to be discussed are whether the chief object of the tubules is the concentration of the fluid produced in the glomeruli, or whether they add to this fluid by a further secretory process, or whether they may not possess both functions, and, in their various parts, alter the fluid flowing through them, either by addition or by withdrawal of water or dissolved constituents. The questions which we have to decide by experiment are :

(1) The nature of the glomerular activity and the conditions which determine the amount of fluid formed by the glomeruli, and, especially, whether or not the energy required for the formation of the glomerular fluid is all furnished by the blood pressure within the capillaries.

(2) The function of the tubules, whether they secrete or absorb, and in either case, what substances ; also what part is played in these processes by the various segments of the tubules, which differ so widely in their histological characters.

FUNCTIONS OF THE GLOMERULI

It is generally agreed that a watery exudation, free from protein, is formed in the glomeruli, and that this becomes concentrated on its way through the tubules, either by the absorption of water and certain salts from it, or by the secretion and addition of urea, uric acid, &c. to it. As to the nature of the glomerular functions, two opinions have been held. According to the Ludwig school, the process is one of simple ultrafiltration, in which, under the pressure of the blood in the glomerular capillaries, the water and crystalloid constituents of the plasma are filtered through the glomerular membrane, leaving the protein constituents behind. This view is now thoroughly vindicated. Heidenhain believed that the process also involved the secretory activity of the glomerular epithelium. If we are to accept the opinion that the only function of the glomeruli is that of filtration, the energy for which is supplied by the blood pressure, certain conditions must be fulfilled. Briefly, they are these :

(1) The fluid passed out from the blood into the Bowman's capsules must be identical with the blood plasma *minus* its colloids.

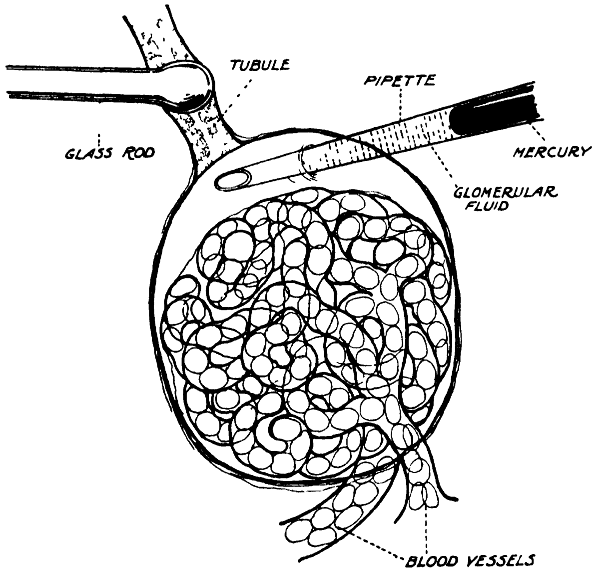
(2) The blood pressure in the glomerular capillaries must be great enough to form the requisite amount of filtrate, and the amount of filtrate formed must vary with that pressure.

There are other requirements also, which follow in the nature of corollaries to these.

One of the difficulties of experimentation on the kidney is that, according to all reasonable theories, the glomerular filtrate must suffer great modification, in volume, in composition, or in both, as it passes along the tubule. The essential problem then is to attempt to disentangle the functions of the glomerular and the tubular portions, and this has been in part solved.

* Fee and Hemingway. *J. Physiol.*, 1928, **65**, 100.
† Borsook and Winegarden. *Proc. Nat. Acad. Sci., Washington*, 1931, **17**, 13.

(1) The Composition of the Glomerular Transudate. There is abundant evidence that the glomerular transudate has a composition identical with that of the plasma *minus* protein. Thus Richards and his colleagues * inserted capillary pipettes into the Bowman's capsule of the frog's kidney, and so obtained directly a small amount of fluid from the capsule (Fig. 590). By micro-chemical methods they showed that the concentration of chlorides, urea, uric acid, phosphate, glucose or various added substances, such as phenol red, etc., in this fluid was approximately equal to that in a plasma filtrate from the same blood. The kidney of *Necturus*, which has very large capsules, was similarly examined by White and Schmitt,† and gave glomerular fluid which resembled that of the frog.



590. Diagram to illustrate Richards' method of obtaining sample of glomerular fluid from Bowman's Capsule in the Frog.

The tubule leading from the capsule is closed by pressing on it with a fine glass rod, operated by a micromanipulator (*v.* p. 14). A very fine glass pipette, connected with a reservoir of mercury, is thrust, by a micromanipulator, through the wall of the capsule, and fluid drawn into it by gradually lowering the mercury reservoir. (Winton and Bayliss's 'Human Physiology.')

The fluid in both these animals contained glucose, which is absent from the urine. Further, the general identity of the glomerular fluid with a plasma ultra-filtrate was shown, for both frog and *Necturus*, by Bayliss and Walker ‡; they showed that the electrical conductivity of the fluid prepared by filtering the blood plasma through cellophane was identical with that of the glomerular fluid drawn by puncture.

The direct evidence obtained from these experiments on amphibian kidneys is so compelling that there is need for little other.

It has been possible to obtain some indirect evidence on the mammalian

* Wearn and Richards. *J. Biol. Chem*, 1925, **66**, 247; Richards and Walker. *Ibid.*, 1930, **87**, 479; Walker. *Ibid.*, 499.

† White and Schmitt. *Science*, 1925, **62**, 334; White. *Amer. J. Physiol.*, 1928, **85**, 191.

‡ Bayliss and Walker. *J. Biol. Chem.*, 1930, **87**, 523.

kidney. Bainbridge and Evans * connected an excised dog's kidney "in parallel" with the arterial side of a heart lung circulation, and obtained a secretion of urine. An artificial pump was substituted for the heart by Hemingway.† The blood-perfused isolated kidney has since been extensively used by Verney and Starling, and by Winton. A constant circulation of warm oxygenated blood can be maintained, the pressure, velocity and composition of the blood supplied to the kidney being under control. The urine obtained from the perfused kidney contains urea, creatinine, and certain colouring matters in higher percentage than they are present in the circulating blood. The urine is, however, hypotonic and contains only

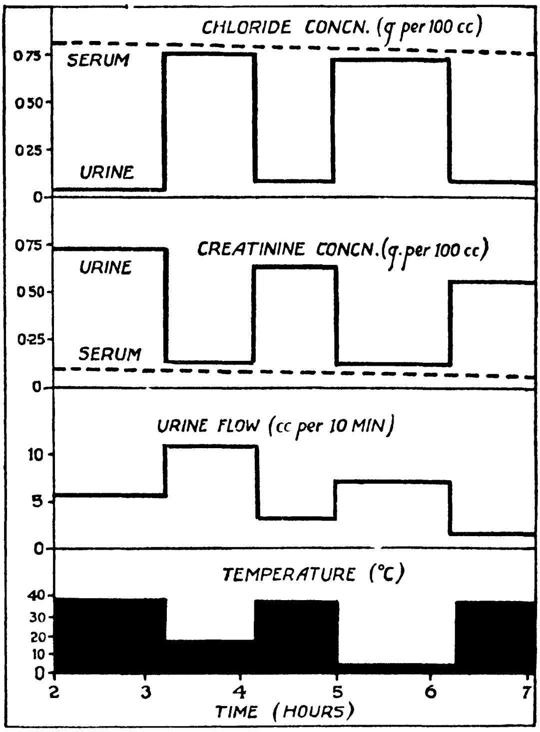


FIG. 591. Effect of Temperature on isolated perfused Dog's Kidney. Cooling put the tubules out of action, so that the urine flow increased, while the chloride content increased and the creatinine content diminished to nearly the same values as in the serum. (BICKFORD and WINTON.)

0·02 to 0·2 per cent. NaCl, as against 0·65 per cent. or more in the plasma of the circulating blood; creatinine, on the other hand, is present in a concentration several times greater than that in the plasma. It is believed that this is because chloride has been absorbed from the glomerular filtrate as it passes down the tubules, while creatinine has been concentrated by removal of water.

If the fluid produced by the glomeruli is similar in its molecular concentration to the circulating plasma, the alterations on its way down the tubules, however effected, must necessitate the expenditure of energy on the part of the cells lining the tubules. Bickford and Winton ‡ subjected

* BAINBRIDGE and EVANS. *J. Physiol.*, 1914, **48**, 278.
† HEMINGWAY. *J. Physiol.*, 1931, **71**, 201.
‡ BICKFORD and WINTON. *J. Physiol.*, 1933, **78**, 14P.

such perfused kidneys to different temperatures and found that when cooled to 25° C. the urine flow was increased, while its composition came very closely to resemble that of the ultra-filtrate of plasma, *i.e.* the chloride increased and the creatinine diminished (Fig. 591). They explained this change as due to the effect of the cold in depressing the metabolism of the tubule cells and so causing a reduction in the absorption of chloride and of water. A similar effect is produced by poisoning the tubules by adding HCN to the blood : the urinary chloride increases and the urea diminishes.*

Even in the normal kidney the constituents of the fully formed urine approximate more closely to the glomerular transudate when the formation is at a rapid rate, because the transudate travels more rapidly along the tubules, so allowing less time for alteration to occur in it, the more rapidly the formation takes place : *i.e.* the quicker the flow of urine, the more nearly does its composition approximate to that of the blood plasma filtrate. The following experiment shows that this is the case. The rate of secretion of a kidney in a heart-lung-kidney preparation was altered by altering the arterial blood pressure. Sodium chloride and urea were added

Time.	Arterial B P. mm. Hg.	Rate of urine flow, c.c per 15 mins.	NaCl content p c		Urea content, p c	
			Serum	Urine	Serum	Urine
1·55	111	14 6	0·86	0·35	0 061	0·352
2·22	147	91·5	0 99	0 69	0·048	0·088
2·30	95	43 5	0·99	0 54	0·042	0·112

to the blood.† It will be observed that as the urine flow accelerates the urine chloride rises and the urea falls, both of which changes bring it nearer in composition to the blood serum.

In accordance with the theory, the total quantity of solids excreted in any given time should rise with any increase in the urinary flow—for whatever the activity of the tubules, the glomeruli must blindly turn out a certain proportion of salts with every cubic centimetre of fluid that they form. Thus, in the above quoted experiment, the following amounts of urea and chloride were excreted per fifteen minutes :

Urine flow, c.c	mg. Urea.	mg. NaCl
14 6	51	51
91·5	81	630
43 5	49	235

Proteins are normally absent from the glomerular filtrate and from the urine, since the glomerular membrane will not allow the normal plasma proteins to pass. It is, however, a very permeable membrane, the pores of which are only just small enough to hold back the normal plasma proteins, the smallest molecules of which are serum albumin with a molecular weight of 72,000.‡ Hæmoglobin, if present owing to hæmolysis, is passed out and appears in the urine if it exceeds a certain small amount in the plasma; its molecular weight is 67,000. If egg albumin, or Bence-Jones protein, or

* STARLING and VERNEY. *Proc. Roy. Soc.*, 1924, **97B**, 321.
† STARLING and VERNEY. *Proc. Roy. Soc.*, 1924, **97B**, 328.
‡ BAYLISS, KERRIDGE and RUSSELL. *J. Physiol.*, 1933, **77**, 386.

gelatin (35,000) are added to the blood they are freely excreted, whereas foreign proteins of high molecular weight, *e.g.* casein (200,000), are not.

Damage to the glomerular membrane by oxygen lack, or in certain diseases, increases the permeability so that the normal serum proteins are excreted, giving rise to the condition of albuminuria. Some persons are at the threshold and pass albumin in the urine when up and about but not when lying in bed (postural albuminuria).

(2) **Factors affecting the Amount of Glomerular Filtrate.** The theory that the fluid formed in Bowman's capsule is an ultra-filtrate of the blood in the glomerular capillaries would lead us to anticipate that the rate at which it is formed in each glomerulus would be determined by factors essentially mechanical in nature. These are the ureter pressure, the venous pressure, the glomerular capillary pressure, the intrarenal pressure (*i.e.* the pressure in the kidney substance) and the extrarenal pressure (*i.e.* the pressure on the outside of the kidney. The resultant of all these is called the *effective filtration pressure.* This would be increased by increase of blood pressure in the glomeruli and opposed by increase of pressure in the Bowman's capsule or ureters.

There are, however, various factors which complicate and obscure this apparently simple relationship. One of these is that the number of glomeruli through which blood is circulating is not necessarily constant. It was shown by Richards and Schmidt * that under certain circumstances, glomeruli of the frog's kidney can be seen to constrict so that no blood passes through them. Intermittent closure of the glomeruli is to some extent spontaneous, but the number so closed is greatly increased by stimulation of the kidney nerves, by various reflexes, and by adrenaline. In the isolated mammalian kidney, adrenaline in very small concentrations causes diuresis with slowing of the blood flow ; in larger amounts it lowers the flow of blood still more and then the urine flow is reduced. These effects may be explained as due to different sensitivity of the afferent and efferent arterioles of the glomeruli. The smaller doses constrict the efferent arterioles and relax the afferent ones, thus raising the glomerular pressure ; larger doses constrict both vessels † (Fig. 592).

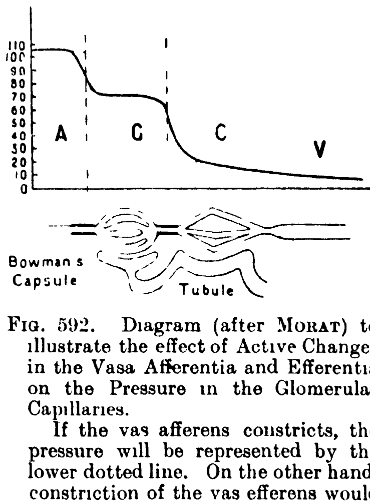


FIG. 592. Diagram (after MORAT) to illustrate the effect of Active Changes in the Vasa Afferentia and Efferentia on the Pressure in the Glomerular Capillaries.

If the vas afferens constricts, the pressure will be represented by the lower dotted line. On the other hand, constriction of the vas efferens would raise the pressure in the glomerulus till it almost equalled that in the renal artery, as is shown by the upper dotted line.

A. Arteries.
C. Tubular capillaries.
G. Glomerular capillaries.
V. Vein.

The urinary secretion can be stopped by obstruction of the ureter. On applying a ligature, the secretion continues for a time, until the pressure in the ureter rises up to a certain point, when the secretion comes to an end. This usually happens when the difference of pressure between urine in the ureter and blood in arteries is between 40 and 50 mm. Hg.

* RICHARDS and SCHMIDT. *Amer. J. Physiol*, 1924, **71**, 178.
† WINTON. *J. Physiol.*, 1931, **73**, 151.

Experiments on the heart-lung-kidney have shown that the effects of alteration of ureter pressure are somewhat complex, but that when the rate of urine formation is changed by alteration of the ureter pressure, the resulting alterations in the composition of the urine are the same as those produced when the rate of urine formation is changed to the same extent by an alteration in the arterial pressure.*

It was objected by Heidenhain † that, according to the filtration hypothesis, a rise of pressure in the renal vein should raise the glomerular pressure and so accelerate the rate of urine formation, whereas, in fact, it retards it. It appears that this is because the effect of the rise of renal vein pressure produces its greatest effect in pressing upon the tubules and so by increasing the intrarenal pressure, retarding the flow of urine in the same way as does a rise of ureter pressure.‡ Whether the urine flow is retarded by raising ureter pressure, raising the venous pressure, or lowering the arterial pressure, however, the resulting alterations in the composition of the urine are practically identical.

Working against the mechanical filtration pressure is the colloid osmotic pressure of the blood plasma. We have seen that the proteins of blood plasma exert an osmotic pressure of about 30 mm. Hg. This implies that ultrafiltration would require a minimum difference of pressure of 30 mm. Hg. between the two sides of the membrane, *i.e.* the pressure in Bowman's capsule, and in the ureter, must be at least 30 mm. Hg. lower than the pressure of the blood in the glomeruli.

The anatomical arrangements are such as to bring the glomerular blood pressure up to a high value. Not only are the vasa afferentia very short, but the vasa efferentia are only two-thirds of the diameter of the vasa afferentia. Moreover, the sudden increase of bed, which ensues when the blood passes from the vas afferens to the bundle of capillaries, must itself diminish the fall of pressure in the latter, or might even cause an actual slight rise, owing to the transformation of the kinetic energy of the moving fluid into potential energy represented by pressure on the walls of the capillaries. It has been shown by White, however, that this effect is small. In the frog, Hayman § has directly estimated the pressures in the various vessels to be as follows :—

Aorta	**37·3 cm. H_2O**	
Afferent arteriole of glomerulus .	**31·0 ,, ,,**	**or 84 per cent. of aortic pressure.**
Capillaries of glomerulus . .	**20·0 ,, ,,**	**,, 54 ,, ,, ,,**
Renal portal vein . . .	**5·2 ,, ,,**	**,, 14 ,, ,, ,,**
Renal vein	**2·6 ,, ,,**	**,, 7·1 ,, ,, ,,**

The colloidal osmotic pressure of the frog's plasma was estimated by White to be about 10 cm. H_2O, which would leave a margin of 4 to 10 cm. H_2O for filtration pressure. Krogh's estimation of the colloidal osmotic pressure of frog's plasma, with collodion of ordinary permeability, was of the order of 6 cm. H_2O, which would leave a still bigger margin.

A method for estimating the mammalian glomerular capillary pressure has been evolved by Winton.|| A pair of kidneys is perfused with blood from the same reservoir : under identical conditions the rate of urine flow is the same from each, and it can be shown, by creatinine clearance, that the rates of glomerular filtration are also equal. Increase of ureter pressure, by introducing a resistance on the ureter, or of venous pressure, by partial clamping of the renal vein, causes a reduction of glomerular filtration rates, and of urine flow : similarly, an increase of arterial pressure increases the

* WINTON. *J. Physiol.*, 1931, **71**, 381.
† HEIDENHAIN. *Hermann's Hdbch. d. Physiol.*, 1883, **5** (I.), 317.
‡ WINTON. *J. Physiol.*, 1931, **72**, 49.
§ HAYMAN. *Amer. J. Physiol.*, 1927, **79**, 389.
|| WINTON. *J. Physiol.*, 1931, **71**, 382 ; **72**, 361 ; **73**, 151 ; EGGLETON, PAPPENHEIMER and WINTON. *J. Physiol.*, 1940, **99**, 135.

rates. Now, if the ureter pressure is increased, and at the same time the arterial pressure is raised, the effects are opposed, and the two pressures can be so altered that no change in the rate of filtration or urine flow results. When this is the case it was shown that,

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in arterial pressure}}.$$

Similarly, if in one kidney the ureter pressure is raised, and in the other kidney the venous pressure is raised, if these pressures are suitably adjusted, so that the changes in glomerular filtration and urine flow are equal in the two kidneys, then,

$$\frac{\text{Absolute glomerular pressure}}{\text{Absolute arterial pressure}} = \frac{\text{Increase in ureter pressure}}{\text{Increase in venous pressure}}.$$

Both these methods indicate a glomerular pressure which is about 0·65 of the arterial pressure.

Again, the venous pressure may be raised in one kidney and the arterial pressure so adjusted in the other that no change of flow occurs, and then, as anticipated from the above,

$$\text{Increase in venous pressure} = \text{Increase in arterial pressure}.$$

The pressure in the Bowman's capsule must be higher than that in the ureter . the exact relationship is uncertain, but the difference between the two probably increases as the rate of filtration rises.

There may thus be, on the two sides of the glomerular membrane, a difference of pressure more than sufficient to cause a filtration of a protein-free fluid from the blood plasma coursing through these capillaries. On lowering the arterial pressure, or on raising the pressure on the tubule side, the filtration ought to come to an end when the pressure in the Bowman's capsule is still 30 to 40 mm. Hg below that in the glomerular capillaries. A number of observers have found that urinary secretion ceases when the arterial blood pressure falls to between 40 and 50 mm. Hg. But if the blood be diluted by the constant infusion of normal saline solution, so as to diminish its protein content, a secretion of urine may be obtained with a blood pressure as low as 18 mm. Hg.

The dependence of the urinary flow on the blood pressure is shown very clearly in some experiments by Richards and Plant * ; they placed a pump in the course of one renal artery of the rabbit, blood clotting having been prevented by hirudin. In this way, the circulation through the kidney was entirely under control, its pressure and velocity being known at any moment. The amount of urine varied directly with the blood pressure and did not depend on the amount of blood flowing through the organ. Similar results have been obtained † in experiments on the heart-lung-kidney preparation and one of these experiments has already been described on p. 1048. The results of such an experiment are shown graphically in Fig. 593, in which it is again shown that when the rate of glomerular filtration is increased by raising the blood pressure, the composition of the urine approaches to that of the serum, *i.e.* chloride increases and creatinine diminishes.

The pressure in the glomerular capillaries can be raised, not only by an increase of general arterial pressure, but also by a relaxation of the afferent arterioles, which would diminish the resistance to the blood flow into the

* Richards and Plant. *Amer. J. Physiol.*, 1922, **59**, 144.
† Verney and Starling. *J. Physiol.*, 1922, **56**, 353.

kidney. Hence, if the nerves entering the kidney be divided, the arterioles of the organ become dilated and the urine flow is increased.* In fact, in many of the older experiments it was shown that, in general, an increase of

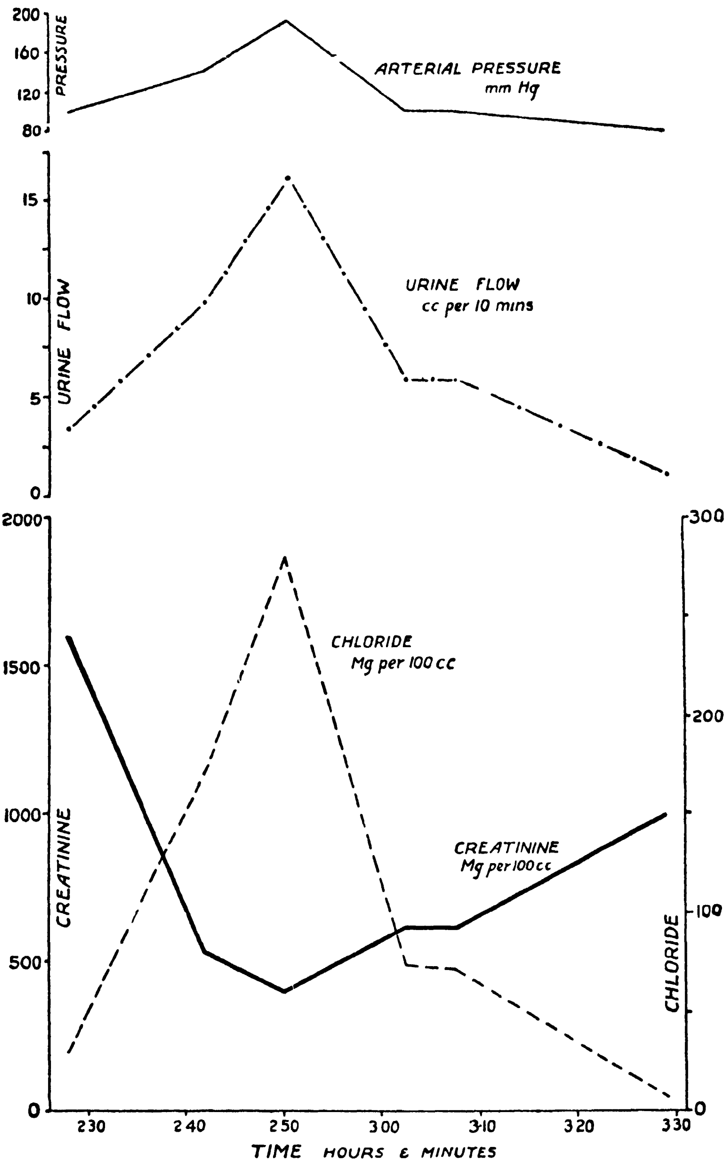


FIG. 593. Effect of Change of Arterial Pressure on Flow and Composition of Urine secreted by an isolated Blood-perfused Kidney. Serum chloride = 760 mg./100 c.c.; serum creatinine = 75 mg./100 c.c. (GILSON and WINTON.)

the volume of the kidney, due to a rise of arterial pressure, and/or to a relaxation of the renal arterioles, accompanied an increase of urine secretion. Conversely, constriction of the renal arterioles, as by stimulation of the

* FEE. *J. Physiol.*, 1929, 68, 305.

splanchnic or renal nerves, led to shrinkage of the kidney and reduction in the urine flow.

All these considerations strongly support the view that the process occurring in the glomeruli is one of filtration, for which the energy is furnished by the blood pressure. It must be remembered, however, that the glomerular capillaries require oxygen for the maintenance of their physical qualities as a filtering membrane. Complete obstruction of the renal artery not only stops the production of urine, but its effects last for some time after the obstruction has been removed, and the first portions of urine obtained when secretion is re-established are found to contain coagulable protein. In the same way, any damage to the glomeruli as a result of defective circulation, of imperfect oxygenation of the blood, or of inflammatory and infective conditions of the kidney, is invariably associated with the passage of protein into the urine.

CLEARANCE TESTS

Information of fundamental value, both theoretical and practical, as regards kidney function can be obtained by means of what are called clearance tests. In order to explain these, let us suppose that a given substance, capable of accurate estimation, is naturally present in, or has been artificially added to, the blood. Further let us suppose that this substance passes an ultra-filter, such as the glomerular membrane is believed to be. The glomerular filtrate will contain that substance in the same concentration as it is present in the blood plasma. Lastly, let us suppose that the substance is neither absorbed from nor secreted into the fluid by the uriniferous tubule, as the stream passes along it. Then all the substance which was contained in the glomerular filtrate will be quantitatively recoverable from the urine.

Now if the urine, of volume V, formed per minute contain U g. per 100 c.c. of the substance, and if the plasma contained P g. per 100 c.c. of it, then it is clear that $\frac{UV}{P}$ c.c. of plasma have been filtered off through the glomerular membrane per minute. This value $\frac{UV}{P}$ is called the *plasma clearance*. It will be evident that all substances which fulfil the above conditions should have the same clearance values; also that substances which are absorbed back into the blood again by the tubules will have lower clearance values, while those which are secreted out from the blood into the tubule will have higher clearance values.

Much work has been devoted to finding substances which fulfilled the above-mentioned requirements and which were either normal plasma constituents, or else were non-toxic. It would appear that the polysaccharide inulin is a suitable substance. Xylose, sucrose and creatinine have also been used, but some xylose and sucrose is reabsorbed, and give low values for clearance, while, in man, some creatinine is secreted by the tubules and so gives too high a plasma clearance. In the dog, rabbit and sheep, creatinine and inulin give identical results. Since the plasma clearance bears a direct relation to the body surface it is often expressed as $C = \frac{UV}{PA}$, *i.e.* in c.c. per sq. m. of body surface, where A = the body surface in sq. m.

Investigations by this method, using creatinine, inulin and ferrocyanide, were carried out by Van Slyke and his colleagues,* on dogs with one kidney removed and the other transplanted to the surface of the body so that

* Van Slyke, Hillier and Miller. *Amer. J. Physiol.*, 1935, 113, 611.

blood samples could be taken and the renal blood flow measured.* They showed that the plasma clearance was about 20 per cent. of the total plasma flowing through the kidney, *i.e.* about one-fifth of the blood plasma was filtered off in the glomeruli. The actual clearance at any time therefore depends on the blood flow through the kidney. In the normal human subject under standard local conditions the plasma clearance is about 122·5 c.c. per min., and varies little (standard deviation 10·7 c.c./min.) in individuals.†

THE FUNCTIONS OF THE RENAL TUBULES

The glomerular transudate, formed, as explained above, in Bowman's capsule, must undergo considerable changes on its way through the tubules. These changes might be brought about either by the addition, to a relatively small volume of the transudate, of a concentrated secretion containing the specific urinary constituents in the required proportions, or else by the reabsorption, from a very large volume of transudate, of water and such other substances as are not to be excreted, so that the fully formed urine represents the unabsorbed residue of the filtrate. These two possibilities, secretion or absorption, would involve the performance of identical amounts of work by the kidney, and there is reason to suppose that both can happen but that in man absorption is normally the more important.

Absorption in the Tubules. According to Cushny, the whole of the changes by which the glomerular filtrate is transformed into urine may be ascribed to processes of absorption occurring in the tubules. He, indeed, denied any fine discrimination to the kidney, and assumed that the composition of the fluid absorbed is always the same, and somewhat resembles Locke's fluid, whatever the needs of the organism at the moment. There would thus be but little absorption of urea and none of sulphate, while the whole of the glucose, a portion of the uric acid and the greater part of the sodium, potassium and chloride are absorbed. According to this view, the constituents of the glomerular filtrate may be divided into two classes, '*threshold substances*' and '*no-threshold substances*,' the former being completely reabsorbed when present in the blood in amount below the 'threshold' value and hence only excreted in the urine so far as their concentration in the blood exceeds that value, while the no-threshold substances are excreted in proportion to their absolute amount in the plasma because they are not reabsorbed at all. Thus, of the threshold substances, the glucose of the plasma is normally below the threshold, and is therefore all reabsorbed and so not present in normal urine. The sodium chloride also comes within the threshold class, but its threshold is usually exceeded in normal conditions, and the excess is then excreted. When the sugar of the plasma rises, as in diabetes, to 0·3 per cent., or when phlorrhizin is given, so as to lower the renal threshold, glucose appears in the urine and then undergoes concentration, just as urea does. It is doubtful whether there are in man any true no-threshold substances as normal constituents of the blood, because most constituents have thresholds, and others are secreted by the tubules to a small extent. The nearest to one appears to be creatinine.

That reabsorption is the dominant function of the tubules is, however, generally conceded. The clearest evidence for it is given by experiments in which it can be directly shown that constituents present in the Bowman's capsule disappear, or are much reduced in amount, during the passage of the

* Rhoads. *Amer. J. Physiol.*, 1934, **109**, 324.

fluid along the tubules. We have already learnt that the fluid drawn by puncture from the Bowman's capsule of the frog contains chloride * ; yet this substance is absent from the frog's urine. Similar results were obtained with *necturus*.† The plasma of the latter animal contains about 0·36 per cent. of NaCl, whereas the urine contains only 0·026 per cent.; when dog's red blood corpuscles were injected into the Bowman's capsule, they were seen to be rapidly washed down into the tubules, by the flow of glomerular filtrate; when the corpuscles entered the tubule they were at once laked, presumably because so much chloride had been abstracted from the fluid that it had become hypotonic. Glucose is likewise present in the glomerular filtrate of frog and necturus, but absent from the urine.

Direct proof of the absorptive functions of the tubules is provided by experiments made in Richards' laboratory, in which the fluid was actually collected and analysed at different points along its course through the tubules. This was effected, in frog and necturus, by the use of capillary pipettes and manipulation under the microscope, as shown in Fig. 594.

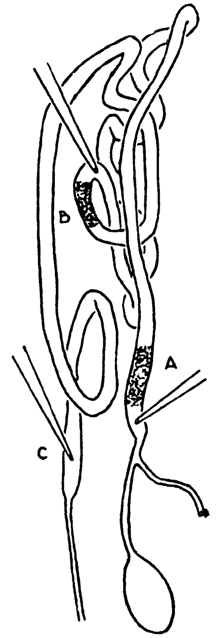


Fig 594. Insertion of micropipettes for collection of tubule fluid from beginning (A), middle (B), and end (C), of a proximal convoluted tubule of *Necturus*. Stippling at (A) represents blockage of lumen by globule of mercury, at (B) by a globule of coloured oil. (Richards. Croonian Lect., *Proc. Roy. Soc.*, 1938, **126 B**, 398.)

Analysis of the fluids collected showed that the chief alterations in total concentration and in Cl occur during the passage along the distal convoluted tubule (Fig 595), and is probably attributable to reabsorption of both water and solids.

In a similar way, creatinine, urea, glucose and phosphate have been followed. Glucose is absorbed completely, and chiefly in the proximal convoluted tubule; after phlorrhizin poisoning, the absorption ceases, and there is then some concentration of glucose by loss of water, which occurs in the distal tubule (Fig 596). As is shown in Fig. 596, inorganic phosphate is also usually concentrated in the distal tubule, but this is again due to absorption of water, and not to secretion. Sometimes phosphate is absorbed back again into the blood; when this occurs it takes place in the proximal tubule, as is normally the case for Na, Cl, and glucose. Since urea in frog and creatinine in necturus concentrate more rapidly than corresponds with removal of water, it may be that these substances can also be secreted by both the tubules.

There is ample support of other kinds for the belief in the reabsorption of water in the tubules. In the first place, the fluid formed in one Bowman's capsule of the frog can be collected and measured, and when multiplied by the number of capsules, gives the total volume of glomerular filtrate formed in the kidney. Thus Richards calculated that if we take 1 c.mm. per hour for the rate of filtration in one capsule (it is usually higher than this), and the number of capsules in a kidney as about 2,000, most of which are probably

* Wearn and Richards. *J. Biol. Chem.*, 1925, **66**, 247.
† White and Schmitt. *Amer. J. Physiol.*, 1926, **76**, 483.

functional under the conditions of the experiment, we should get 2 c.c. of glomerular filtrate formed per hour; but the amount of urine formed per hour under the same conditions is rarely more than 0·2 c.c., so that we must conclude that 90 per cent. of the water passed out into the capsules is reabsorbed in the tubules.

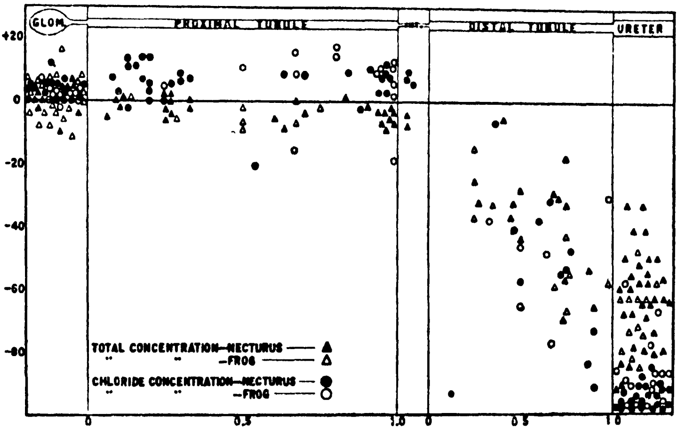


FIG 595. Chart showing differences between blood plasma and fluid collected from various points on renal tubules, with respect to total solids and Cl concentration. Zero line represents plasma. Cl scale shows per cent. difference from plasma. Concentration of solids represents change in length of column of fluid equilibrated by Barger's method (Osmotic pressure) against plasma. (RICHARDS. Croonian Lect., 1938.)

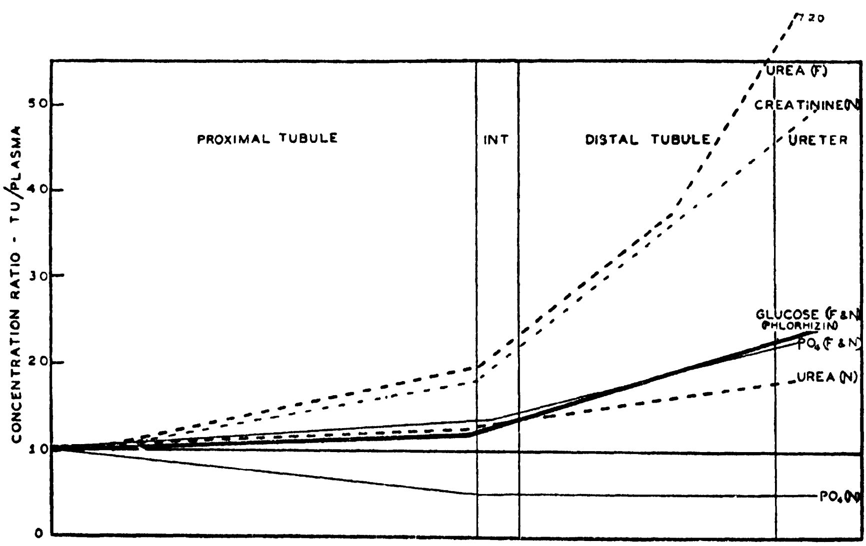


FIG. 596. Average curves of changes in concentration of urea, inorganic phosphate, and creatinine in passing the tubules in frog (F) and *Necturus* (N). Also changes in glucose after phlorrhizin. (RICHARDS. Croonian Lect., *R.S.*, 1938)

Most of the experiments on the excretion of dyestuffs by the kidney are now believed to be explicable as due to active secretion.* Proof that the tubules can effect the absorption of water, has been provided by Richards and his colleagues by perfusion of

* MARSHALL and VICKERS. *Johns Hopkins Hosp. Bull.*, 1923, 34, 1.

the frog's kidney. The blood vessels of the tubules, as will be shown, can be perfused from the renal portal vein, and those of the glomeruli from the aorta. If the Bowman's capsules be filled with dilute phenol red solution by injecting it directly with a micropipette into a selected capsule, and the arterial perfusion be stopped, while the perfusion of the renal portal vein be continued with saline solution containing no phenol red, the dye will nevertheless be seen to become concentrated in the tubules along which it passes.* Even when an excised frog's kidney is immersed in a dilute solution of phenol red, the dye is found to be concentrated in the tubules, which are conspicuously stained. This may in part have been caused by active absorption (secretion) of the dye by the tubule, but is explained by Richards and Barnwell as being due to the taking up of the dilute dye solution as a whole by diffusion into one part of the tubule, and its concentration, by withdrawal of water, at another portion. Addition of cyanide to the solution, though not preventing the diffusion of phenol red into all parts of the kidney, does prevent its concentration locally in any part of the tubules, with the result that, after the soaking in cyanide dye solution there is merely a diffuse staining.

We may accept, as the balance of evidence, presented here in somewhat biassed form for the sake of simplicity, that there is reabsorption of water and of such dissolved constituents as are to be retained in the body, from the glomerular transudate as it passes on its way down the tubules. The dissolved constituents are reabsorbed to different extents; creatinine little, if at all, and urea not greatly; chloride, glucose and phosphates more than sulphates.†

The power of absorption possessed by the cells of the tubules is not indefinitely large, and the urine can therefore never exceed a certain concentration, at which its osmotic pressure just equals the absorptive power of the cells. This limiting concentration differs in different animals, the cat being able to absorb against a resistance of fifty to sixty atmospheres, while the human kidney cannot concentrate against a resistance of more than twenty-five atmospheres. The presence of any difficultly absorbable substances in the glomerular filtrate, *e.g.* urea, sodium sulphate, or phosphate, must therefore limit the absorption owing to the osmotic resistance they offer to the absorptive powers of the cells. These substances will therefore act as diuretics. In the same way the threshold substances will act as diuretics, provided that they are present in the plasma in proportions above the threshold, so that they can no longer be absorbed by the cells of the tubules.

Secretion in the Tubules. Histological examination of the kidney tubules shows a great diversity of structure in the epithelium with which they are lined. Hence, it is quite conceivable that some portions of the tubule might absorb certain substances from the filtrate while other portions were capable of secreting: or the same cells might even absorb certain things and secrete others. Yet for one or two decades, physiologists appeared to be so satisfied with the evidence in favour of reabsorption as to forget that the demonstration that urine formation could not be explained wholly on the filtration-secretion hypothesis by no means put that method out of court as an accessory.

Once an instance had been produced of urine formation in which secretion indubitably played a part, the door was open to admit secretion as a possible collaborator with absorption, in the modification of the glomerular filtrate. Such an instance was produced when Edwards, Marshall and Homer Smith,‡ recalling the fact that certain fish had kidneys from which glomeruli

* Richards and Barnwell. *Proc. Roy. Soc.*, 1928, **102B**, 72; Richards. "Methods and Results," etc., 1929, p. 34.

† Rehberg. *Bioch. J.*, 1926, **20**, 447, 461.

‡ Edwards. *Anat. Rec.*, 1925, **44**, 15; Marshall. *Bull. Johns Hopkins Hosp.*, 1929, **45**, 95; Marshall and Homer Smith. *Biol. Bull.*, 1930, **59**, 135; Marshall. *Amer. J. Physiol.*, 1930, **94**, 1.

were absent, pointed out that urine formation must in those species be a purely secretory phenomenon. Their further investigations on these aglomerular kidneys of at least twenty-five species of fish belonging to five different families have, more than anything else, provided the faith that was required for acceptance of the possibility of tubular secretion.

It is suggested that the explanation of the differences in kidney structure in different species of animals is bound up with their phylogenetic history, the glomerulus being developed when water was abundant, and reabsorption then being necessary to prevent loss of valuable substances; when water was scarce kidney function could be carried out either by secretion alone, the glomeruli being lost, or by reabsorption with or without the addition of secretory power, the glomeruli being retained.

When the possibility of secretion by the tubules had been demonstrated, it was natural to re-examine the classical experiments of the 'seventies, which had once been thought to provide evidence in its favour, and which had in the meantime been explained in other ways. Heidenhain,* for instance, attempted to throw light on the excretory functions of the kidney by studying the mechanism by which it excretes sodium sulphindigotate ('indigo carmine'). If the dye be injected into the veins, it is excreted in a concentrated form by the kidney, so that the urine assumes a dark blue colour. If the animal be killed when the excretion of the pigment is at its height and the kidneys be rapidly fixed with alcohol, all parts of the kidney present a blue colour, which is especially marked in the medulla. Under these circumstances, the fluid formed in the glomeruli rapidly carries down the dyestuff, wherever it may be turned out, into the tubules of the pyramids. In order to discover the exact locality of the cells involved in its excretion, we must stop the fluid current by some means. This can be effected in two ways, viz. by section of the spinal cord in the neck, so as to reduce the blood pressure to about 40 mm. Hg, *i.e.* below the minimum necessary for the production of urine, or by cauterising portions of the surface of the kidney by means of silver nitrate. If the indigo be injected into the veins after section of the cord, and the animal be killed half an hour later, the colour in the kidneys is seen to be confined to the cortex, and, on making sections, granules of the pigment are found within the lumen and in the cells of the convoluted tubules. If part of the surface of the kidneys has been cauterised, the stain is confined to the convoluted tubules of the cortex only under those areas in which the glomerular functions have been abolished by cauterisation.

It can be, and is, objected to these results that they may equally well be brought about by absorption and not by secretion. If indigo carmine is turned out by the glomerulus, it will be in so dilute a solution that the glomerulus will not be stained. As the transudate descends the tubules it undergoes concentration, leading to precipitation of the dyestuff in the lumen of the tubules, and the granules in the cells may be the result, not of secretion but of absorption. This objection applies to many of the experiments carried out with dyestuffs, particularly those performed on mammals. Some experiments by Marshall, however, certainly point to the active secretion of phenol red by the dog's kidney. Phenol red is partly held in the plasma in combination with protein, and hence is only partly separable by ultra-filtration, 75 per cent. of it remaining behind. Yet the amount of phenol red passed out in the urine was so large that in order to explain it by reabsorption, the blood flow through the kidneys would have to be over 1 l. per minute, even if half the plasma filtered through

* HEIDENHAIN. *Pflüger's Arch.* 1875, **9**, 1; *Arch. mikr. Anat.*, 1874, **10**, 30.

and was reabsorbed. Hence the phenol red must have been torn away from its colloidal union, either by secretion in the tubules, or, which is hardly likely, by active secretion at the glomerular membrane.

Among other substances foreign to the blood, the organic iodine compounds "diodrast" and "hippuran," which are used for X-ray studies of the kidney, are also secreted by the tubules.

By taking advantage of the peculiarities of the renal circulation in amphibia, claims of another kind have been made for the possession of secretory functions by the cells of the tubules. A number of renal arteries leaving the aorta enter the kidney and supply the whole of the glomeruli, the vasa efferentia from which pass, as in the mammalian kidney, into the intertubular capillaries. These, however, also have connections with the renal portal vein, shown in the Fig. 597, and it is certainly possible, by perfusing the renal portal vein at a high pressure, to cause a flow in that direction. If all the renal arteries be ligatured, the glomeruli were stated by Nussbaum * to have been entirely cut out of the circulation, though the tubules still receive venous blood through the renal portal vein. Nussbaum found that ligature of all the renal arteries caused cessation of the urinary secretion, which could be reinduced by injection of urea. He concluded that urea, with water, was secreted by the tubules. This experiment has often been repeated, with modifications,† and with similar results. Of recent years, there has been some difference of opinion as to the accuracy of the anatomical basis of them. Hayman,‡ and Bensley and Steen,§ however, after the most careful enquiry, consider that Nussbaum's original description is substantially correct.

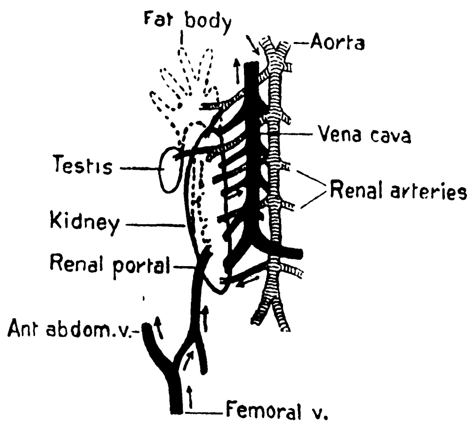


FIG. 597. The Vascular Supply to the Kidney in the Frog.

Experiments by Bensley and Steen on the excretion of dyes by the frog's kidney, suggest that these are actually secreted by the cells of the proximal convoluted tubule, and that there is absorption of water, and perhaps of threshold substances, in the distal convoluted tubule. In these experiments, the kidneys were observed under the microscope at intervals after the injection of indigo carmine or phenol red. Some of the renal arteries were tied, and the nephrons examined were observed to have no glomeruli functioning. In these, the dye was seen to appear in the cells of the proximal tube in vacuoles, *which then passed out* into the tubule. When indigo carmine was given first, and phenol red after it, the first dye was displaced inwards into the lumen by the second, which followed it along the whole tube, both becoming concentrated as the distal tubule was traversed. Similar results have been obtained by observation under the microscope, on frog's kidneys with glomeruli put out of action by ligature of the renal artery, and on rat's kidneys in which glomerular circulation was stopped by section of the spinal cord. The dye used was fluorescin, which was rendered visible by ultra-violet light.‖

To sum up, it seems probable that secretion, when it occurs, is effected by the proximal convoluted tubule, which is also able to absorb water and

* NUSSBAUM. *Pflüger's Arch.*, 1878, **16**, 139; **17**, 580.
† BAINBRIDGE and BEDDARD. *Bioch. J.*, 1906, **1**, 255; ATKINSON, CLARK and MENZIES. *J. Physiol.*, 1921, **55**, 253.
‡ HAYMAN. *Amer. J. Physiol.*, 1928, **86**, 331.
§ BENSLEY and STEEN. *Amer. J. Anat.*, 1928, **41**, 75.
‖ ELLINGER. *J. Physiol.*, 1940, **97**, 433.

certain other constituents, such as glucose and salts. Concentration by withdrawal of water is effected chiefly by the distal convoluted tubule.

Secretion of the usual urinary constituents does not seem to occur to any great extent in the mammal, though in man some creatinine, and in birds and reptiles uric acid, are definitely excreted. Unusual substances, such as dyes, and diotrast and hippuran, are certainly excreted by mammalian tubules.

Urea Clearance. We have seen that the plasma clearance test may be used as a means of assessing the volume of glomerular filtrate, provided that the substance chosen for estimation is neither absorbed nor excreted by the tubules. As measured in this way, inulin gives clearances of the order of 123 c.c. per minute. Urea, on the other hand, gives values of only about 75 c.c. per min., so that there is apparently some reabsorption of this substance. If the urea level of the blood is raised by taking urea by the mouth the clearance is unaltered, *i.e.* the same fraction of the urea is removed. But if the number of functional glomeruli is reduced by reduction of the blood flow through the kidney, or by disease, the clearance is diminished. In some cases of nephritis it may be only 4 or 5 c.c. per minute.

To carry out a urea clearance test the subject drinks about a pint of water in order to get a good diuresis. The bladder is emptied to begin with, and again after one hour and two hours, and the volume V in c.c./min. is noted. A blood sample is taken at one hour, and the urea content of blood (B) and urine (U) are determined. The clearance $C_{ur} = \frac{UV}{B}$ c.c./min. or, in percentage of the normal clearance,

$$C_{ur\,p\,c} = 1{\cdot}33\,\frac{UV}{B}$$

If the urine flow is less than 2 c.c./min. the formula is :

$$C_{ur\,p\,c} = 1{\cdot}85\,\frac{U\sqrt{V}}{B}.*$$

Renal Blood Flow. Diodrast is apparently completely removed from the blood flowing through the kidney. Hence its plasma clearance will be equal to the flow of plasma through the kidneys, so that if the plasma and corpuscle content of the blood are also known (by hæmatocrite measurements), the blood flow can be deduced. It has been found in this way that the effective circulation through the kidneys of an average man of surface area of 1·73 sq. m. is 1,384 c.c./min. or about 30 per cent. of the cardiac output.

Various functional tests for the efficiency of the kidney have been proposed for clinical use. Most of them depend on the determination of the degree of concentration in the urine of substances present in or added to the blood, or of clearances of various constituents. Books on clinical chemistry should be consulted for an account of these tests.†

THE ADAPTATION OF THE RENAL FUNCTIONS

On its way down the tubules the glomerular filtrate undergoes extensive alteration in character, some of its constituents, water, salts and sugar, being absorbed. Certain other constituents such as urea, uric acid, creatinine, and

* PETERS and SLYKE. Quantitative Clinical Chemistry, II., 1932, p. 564. (Williams and Wilkins, Baltimore.)

† HARRISON. "Chemical Methods in Clinical Medicine," 1930, p. 56 (Churchill, London)

probably phosphates and ammonia, are not reabsorbed to any great extent, but some of them may even be added to it, so that we finally obtain the urine. When we examine the urinary secretion in the intact animal, we cannot but be struck with the manner in which its amount and concentration are adapted to the needs of the body as a whole. The function of the kidney is to preserve the normal composition of the body fluids, by turning out those substances which are abnormal or present in too great an amount. The behaviour of the tubule cells with regard to any given substance will therefore depend to a certain extent on the previous nutritive history of the body.

If, for instance, in consequence of the administration of sodium chloride in large quantities to the animal during the few days preceding the experiment, the body is overloaded with this salt, it becomes an abnormal constituent and the kidney secretes a urine far richer in sodium chloride than is the blood plasma. Moreover, when diuresis is produced in such an animal by the injection of equivalent quantities of sodium chloride and sodium sulphate, there is no diminution of the NaCl in the urine towards the end of the diuresis, but its percentage rises steadily as the rate of urinary flow diminishes. On the other hand, a total deprivation of sodium chloride extending over several days, although not altering to any large extent the percentage amount of this salt in the blood plasma, leads to a total disappearance of the salt from the urine, the whole of the sodium chloride present in the glomerular transudate being reabsorbed on its way through the urinary tubules. When large quantities of water are taken by the mouth, there is a rapid excretion of a very dilute urine, so that the constitution of the body, and of its fluids, is but little altered by the water ingested. The kidney plays an important part in the regulation of the hydrogen ion concentration of the blood. When there is increased production of acids in the body, as occurs on a large protein diet, or under abnormal conditions, such as diabetes, the excess of acid is excreted in the urine, which thus becomes more acid. If, in spite of this, the bicarbonate of the plasma is lowered, less chloride is eliminated, so that the osmotic pressure of the plasma is unaltered. Alkaline salts taken by the mouth, or formed in the body as a result of the oxidation of the salts of the vegetable acids, cause the secretion of an alkaline urine. Normal urine contains no sugar, although this substance is an unfailing constituent of the blood plasma. If, however, the sugar in the blood rises by a small amount, namely, to 0·2 per cent., the excess of sugar is at once excreted in a highly concentrated form by the kidneys, so that this substance may occur in the urine to the extent of 6 or 7 per cent.

The simplest explanation of the varying activity of the kidney would be to assume that this organ reacts to very minute changes in the composition of the circulating blood. For instance, Clark * perfused the arterial system of the frog with Ringer's solution containing 0·1 per cent. of glucose, and at the same time supplied the tubules *viâ* the renal portal vein with Ringer's solution of varying glucose content. When this solution contained up to 0·1 per cent. of glucose, all the glucose passed out into the Bowman's capsule was reabsorbed, so that none was present in the urine; when it contained more than 0·1 per cent. there was less and less reabsorption as the amount supplied to the tubules was increased. Thus the rate of reabsorption of glucose was determined by the glucose content of the fluid perfused through the tubular capillaries.

* Clark. *J. Physiol.*, 1922, **56**, 201.

Adaptation to Reduction of Kidney Tissue. The adaptability of the kidney in performing its functions is well seen by the effect of removal of a portion of the kidney, or of deprivation of the blood supply to part of it by ligature of one of the branches of the renal artery. In either case the kidney thus reduced secretes no less urine than the other kidney, which remains in possession of all its functional parts.* We may suppose this to be due to the fact that in ordinary circumstances not all the functional units of the kidney are in action. Each human kidney, according to Traut,† contains about 4½ million glomeruli. If we suppose that the 9 million units represented by the two kidneys were each forming only 1 c.mm. of glomerular transudate per hour (*i.e.* as in the frog), and that 90 per cent. of that transudate was reabsorbed, this would still give a volume of 900 c.c. of urine per hour. Since, on the average only 60 c.c. of urine are formed per hour, it is highly improbable that in ordinary circumstances more than about 10 per cent. of the glomeruli and tubules will be in action at the same time. There is thus a considerable margin of reserve in the kidney.

It has been found that when kidney tissue is considerably reduced, as, for example, by excision of one kidney, or by removal of one kidney and half the other, the volume of urine excreted may not only not be diminished, but may even be increased,‡ the excreted urine being of a dilute type more nearly representing the glomerular transudate. These results have been obtained also on the heart-lung-kidney preparation. Experiments were made by Verney and Pickford § in which the two kidneys of the dog were separately perfused from a heart-lung preparation. ‖ Under similar conditions the two kidneys secreted identical urines at the same rates. When part of one of the kidneys was thrown out of action by ligature of one of the branches of the renal artery, it secreted a dilute urine, and often at a greater rate than the other kidney. This is probably due to the effect of reduced intrarenal pressure.¶ The increased urine production of the remaining units is accompanied by their failure to concentrate the urine formed, because of its more rapid transit along a relatively small number of tubules.

A similar condition is seen in pathological states where there has been destruction of a large amount of kidney tissue, and Verney has appropriately compared this with the dilatation seen in a failing heart during exercise ; the remaining kidney tubules have, so to speak, become worked to the limit of their functional reserve. We therefore have two types of reserve in the kidney tissue—structural reserve by the closing down of those glomerulo-tubular units whose services can be temporarily dispensed with, and, secondly, the functional reserve represented by the extent to which the tubules, in effecting their work of concentration, are working to the limits of their capacity.

DIURESIS

An increase in the rate of urine formation is called diuresis. It may be caused by copious water drinking, by pathological states, or by the action of certain drugs.

Two types of factor might be concerned in promoting an increased flow of urine. These are :

* VERNEY. *Lancet*, 1930, (ii.), p. 63.
† TRAUT *Contrib. to Embryology*, Washington, 1923, **15**, 103.
‡ BRADFORD. *J. Physiol.*, 1899, **23**, 415.
§ VERNEY. *Lancet*, 1929, March 30th, July 12th.
‖ VERNEY, WINTON and CANNY. *J. Physiol.*, 1930, **68**, 333.
¶ WINTON. *J. Physiol.*, 1934, **82**, 27P.

(1) Increased glomerular transudation from mechanical causes, *e.g.* rise of pressure in the glomerular capillaries, reduction of protein content of the blood, or the opening-up of glomeruli previously closed by constriction.

(2) Reduced absorption in the tubules, such as may, conceivably, happen when the diuretic substance is not absorbed, or when the pituitary hormone is reduced.

Clues to the way in which diuresis is brought about are provided by the study of the osmotic work, oxygen usage and so, from these, of the efficiency of the isolated kidney.

Pressure Diuresis. When diuresis is caused by an increase of arterial pressure, it would be expected that if the diuresis was only due to increased glomerular filtration, and not at all to altered tubular absorption, there would be no increase in the oxygen usage of the kidney, and no change in its efficiency. Actually, however, a rise of arterial pressure does cause an increase, not only in the filtration (creatinine clearance), but also in oxygen usage, osmotic work and efficiency.* Doubling the urine flow in this manner causes about 40 per cent. increase in osmotic work and 25 per cent. in efficiency. We may infer, therefore, that even in this apparently simple case of increased glomerular activity, there is a concomitant increase in tubular reabsorption. The concentrations of all the constituents of the urine during pressure diuresis move towards their concentration in the plasma, so that the work done per unit volume of urine produced is less, but owing to increased work on the part of the tubules, the work done per unit of time is raised. These facts were discovered by the use of the isolated kidney.

Dilution Diuresis. When the plasma colloids are diluted by the addition to the blood of Ringer's solution, the effective filtration pressure and glomerular filtration rate are raised. When compared with the effect of an equivalent increase of effective filtration pressure by raising the arterial pressure, however, the effect of dilution is found to be six times as great in isolated kidneys and fifteen times as great in intact anæsthetised animals.† This appears to be due to an increased permeability of the glomerular membrane in the dilution diuresis, but the osmotic work is also increased, viz., about 40 per cent. when the flow of urine is doubled. In the intact dog there is also diminished reabsorption of water.

Water Diuresis. When a large amount of water is taken by the mouth, the change in the composition of the blood plasma may be very slight, and mainly due to loss of electrolytes diffusing out into the intestine and tissues, though the kidney is secreting large quantities of extremely dilute urine.‡ This is believed to be due to a reduction in the reabsorption of water by the tubules, rather than to an increase in glomerular filtration. Even when dilution of the plasma does occur, the maximal rate of urine formation lags some 20 to 30 minutes behind the maximal dilution,§ so that it is not certain that the kidney is responding directly to the increased water content or lowered osmotic pressure of the blood.|| It is possible to bring about a lowering of molecular concentration by injecting water or a hypotonic salt solution directly into the blood. Under these circumstances there is increased

* EGGLETON, PAPPENHEIMER and WINTON. *J. Physiol.*, 1940, **97**, 363.
† EGGLETON, PAPPENHEIMER and WINTON. *J. Physiol.*, 1940, **98**, 336.
‡ HALDANE and PRIESTLEY. *J. Physiol.*, 1916, **50**, 296; RIOCH. *J. Physiol.*, 1930, **70**, 45.
§ SMIRK. *J. Physiol.*, 1933, **78**, 113, 127, 147.
|| BALDES and SMIRK. *J. Physiol.*, 1934, **82**, 62.

secretion of urine,* but again only after considerable delay.† In the same way, when a large quantity of sodium chloride is taken, the result is a temporary rise in the salt concentration of the plasma which may last a day or two. This is then succeeded by an increased excretion of salt by the kidney, but this occurs at a period after the salt concentration of the plasma has returned to normal. The salt ingested seems at first to pass into the tissues, and is only got rid of from these by an increased excretion after a considerable latent period.

It is a curious fact that if the water reserves of the body are reduced in thirst, the administration of the amount of water lacking from the body produces a normal diuresis ‡ after the usual lag. It is not clear, therefore, exactly what it is that the kidney does respond to when water is drunk, but the suggestion is that it is a hormone effect.

We have seen that the isolated perfused kidney secretes urine containing only traces of sodium chloride, although the content of this salt in the blood serum is rising steadily throughout the experiment. Addition of a small

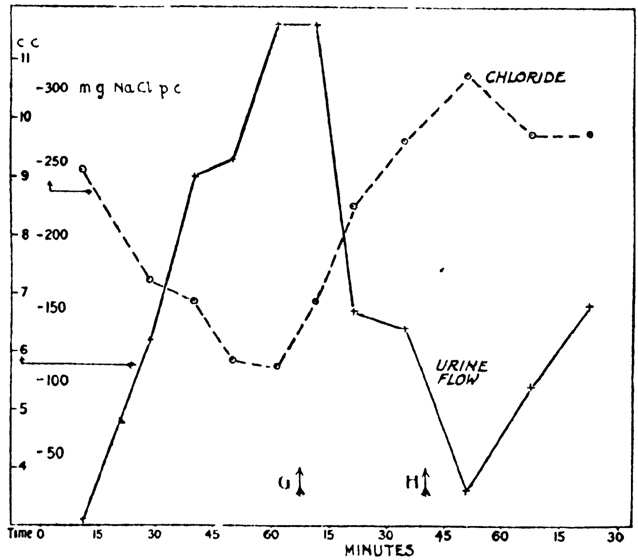


FIG. 598. Volume (in c.c. per 15 min.) and Chloride Content of Urine (as mg. NaCl per cent.) of Dog's Kidney in Heart-Lung-Kidney Perfusion. At G a dog's head was switched into, and at H out of, the circuit. (VERNEY, *Proc. Roy. Soc.*, 1926, **99**, 487.)

amount of extract of the posterior lobe of the pituitary body at once reduces the volume of the urine and increases the amount of chloride in it.§ If, in such a perfusion, the blood is also circulated through a head, the volume of urine is speedily reduced, and its chloride content correspondingly increased (Fig. 598), but this does not occur if the pituitary body has first been removed from the head.

In the disease known as *diabetes insipidus* the patient passes large quantities of watery dilute urine and suffers very intense thirst as a

* FEE. *J. Physiol.*, 1929, **68**, 305.
† NEWTON and SMIRK. *J. Physiol.*, 1933, **78**, 451.
‡ HELLER and SMIRK. *J. Physiol.*, 1932, **76**, 1.
§ STARLING and VERNEY. *Proc. Roy. Soc.* 1925, **97B**, 321.

consequence. Diabetes insipidus is often associated with demonstrable alterations, of a destructive nature, in the pituitary body, probably in its posterior lobe.* Excision of the pituitary body may sometimes, though by no means regularly,† for a time, at any rate, bring about a similar condition, namely, increased formation of urine and disappearance of chlorides from the urine ‡; and it is interesting to note that injection of pituitrin into a patient with diabetes insipidus, § or addition of a small amount of the extract of the posterior lobe of the pituitary body to the blood circulating through the excised kidney, suspends the condition, the urine diminishing in amount while the chlorides in it rise considerably and in some cases even exceed the percentage amount of chlorides in the plasma. These and similar facts remind us that the kidney is not an isolated organ, guided in its activity merely by the nervous system, through its influence on the blood vessels, or by the composition of the blood, but that it is played upon by hormones, which reach it from different parts of the body according to the chemical condition in which these parts happen to be.

It has been suggested that water diuresis may be due to suspension of liberation of the pituitary secretion into the blood, the delay in onset of diuresis being occasioned by the slow rate of destruction of the hormone. ‖ Although this theory is attractive, it is embarrassed by the fact that a normal water diuresis can be elicited in animals deprived of the pituitary.¶

Action of Diuretics. Attempts have been made to illuminate the problem of renal secretion by studying the action of diuretics, *i.e.* substances which, injected into the blood stream or absorbed from the alimentary canal, increase the secretion of urine. A large increase in the urinary flow can be brought about by the intravenous injection of saline diuretics such as sodium sulphate or potassium nitrate, or neutral crystalloids such as urea or sugar.

When a concentrated solution of salt is injected into the circulation, the total volume of the circulating fluid is soon increased by the addition to it of water drawn from the tissues, *i.e.* a condition of hydræmic plethora is set up, just as if a large bulk of normal saline fluid had been injected into the circulation. The kidney placed in an oncometer shows a great increase in volume. While the plethora lasts, there are mechanical conditions at work in the kidneys, *i.e.* rise of pressure, greater rate of flow, and diminished concentration of plasma—all of which would concur in producing an increased glomerular transudation. With certain salts, such as sodium chloride, the diuresis may be coterminous with the hydræmic plethora, but with other members of this class, such as glucose, the diuresis always outlasts the plethora, so that the continued augmentation in the secretion of urine leads to an actual concentration and diminution of the volume of the circulating blood. As we have already seen, every substance which is not absorbed from the glomerular filtrate by the tubules should act as a diuretic, since it will oppose osmotic resistance to the absorbing powers of the cells. Thus the low-threshold substances, urea, and sodium sulphate, nitrate, and phosphate, will, in any concentration, act as diuretics. The threshold substances will produce diuresis so long as their concentration in the plasma surpasses their normal threshold value.

* Frank. *Berl. klin. Woch.*, 1912, **49**, 393.
† Fee. *J. Physiol.*, 1929, **68**, 39, 305.
‡ Vassale and Sacchi. *Riv. Sper. di Frenist*, 1892, **18**, 525.
§ von den Velden. *Berl. klin. Woch.*, 1913, **50**, 2083.
‖ Klisiecki *et al.* *Proc. Roy. Soc.*, 1933, **112B**, 496.
¶ Newton and Smirk. *J. Physiol.*, 1934, **81**, 172; White and Heinbecker. *Amer. J. Physiol.*, 1938, **123**, 566.

In urea diuresis, the glomerular filtration is almost unaltered, as is shown by the creatinine clearance ; the osmotic work is increased, but the oxygen consumption is not, *i.e.* the efficiency is increased. For a doubling of urine flow in the isolated kidney the osmotic work is increased 65 per cent. and the efficiency 60 per cent.* by urea diuresis.

An important factor in regulating glomerular activity is the contractility of the afferent and efferent arterioles of the glomeruli, already referred to, and in consequence of which the number of patent glomeruli may vary.

With specific diuretics, such as caffeine, the position is not quite so clear. In most cases, injection of caffeine in the rabbit brings about a dilatation of the kidney and a proportional increase in the secretion of urine. But cases have been recorded in which expansion of the kidney occurred without any increase in urinary flow, and, on the other hand, augmented urinary flow without any increase in the kidney volume. The general rule is that a greater rate of blood flow is obtained *pari passu* with the increased urinary flow ; but a consideration of certain peculiarities in the renal circulation must prevent us from laying too much stress on apparent exceptions to the rule. A dilatation of the afferent vessels, and a slight constriction of the efferent vessels, would cause a considerable rise of pressure in the glomerular capillaries, and a consequent increased transudation, without necessarily altering to any marked extent the total circulation of blood through the whole organ.

Hayman and Starr † have counted in the rabbit's kidney the number of glomeruli which were open to circulation when in the normal state, and also after administration of caffeine. They find that, whereas in the normal state, from 50 to 90 per cent. of the glomeruli are patent, all of them are opened after a dose of caffeine. After adrenaline, on the other hand, only 10 per cent. of them may be in use, and the urine flow much reduced.

On the other hand, it seems probable that many diuretics—of which caffeine may be one—act by altering the activity of the tubules. We may assume that any action these substances possess on the tubules is one of paralysis, complete or partial, of their powers of absorption. Thus the action of phlorrhizin may be assumed to paralyse the absorptive powers of the tubular cells for glucose—*i.e.* to reduce glucose for this particular kidney to the state of a no-threshold substance. The glucose in the glomerular transudate, in passing through the tubules, may thus be concentrated sixty to a hundred times. Since glucose is made in the body, and supplied to the circulating blood at such a rate as will maintain its concentration in the plasma at a definite height, the loss of sugar in the urine will be continued, and the percentage in the plasma will not tend to diminish progressively with the increased secretion of urine, as would occur, for example, in the case of urea. We may assume that different diuretics have similar powers of paralysis on the absorptive mechanisms of the tubules, either general, or confined, as in the case of phlorrhizin, to one or other of the normal constituents of the plasma. In the same way, pituitrin appears to depress the chloride-absorbing functions of the tubules while exalting their powers of water absorption.

Renin. Saline extracts of alcohol-dried kidneys contain a substance which raises arterial blood pressure and accelerates urine formation owing to reduction of tubular reabsorption of water and sodium chloride.‡ The active substance called renin, is a protein, and it has been suggested that it plays a part in the production of the rise of

* Eggleton, Pappenheimer and Winton. *J. Physiol.*, 1940, **97**, 363.
† Hayman and Starr. *J. Exp. Med.*, 1925, **42**, 641.
‡ Pickering and Prinzmetal. *J. Physiol.*, 1940, **98**, 314.

blood pressure, with diuresis, which is a feature of certain kidney diseases. Reduction of the blood flow to a kidney, by clamping, leads to a state of hypertension similar to the pathological condition, and is attributed to release of renin or some related substance into the blood stream. The release, into the blood, of a vasoconstrictor substance, by isolated perfused kidneys has been demonstrated.*

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3. THE PHYSIOLOGY OF MICTURITION

The urine as it is formed passes through the ureters to the bladder, where it gradually accumulates, and is voided at intervals.

THE URETERS enter the bladder at its base, at the two posterior angles of the trigonum. Their entrance is by an oblique valvular orifice which prevents reflux of urine from bladder to ureter. The plain muscular coats of the ureter show rhythmic contraction waves; these pass from the pelvis of the ureter to the bladder at a velocity of about 2 to 3 cms. per second, driving the contained fluid in front of them, so that the pelvis of the ureter is emptied of fluid. The intermittent injection of urine into the bladder as each wave of contraction reaches it can be seen on cystoscopic examination in man. The frequency of the contractions, normally about three per minute, is increased by warming the ureter, and by distension, so that the waves are more frequent when the secretion of urine is profuse. The ureters are supplied with nerve fibres from the splanchnic nerves by way of the renal plexus, and at their lower ends from the hypogastric nerves. Stimulation of the hypogastrics closes the openings into the bladder.

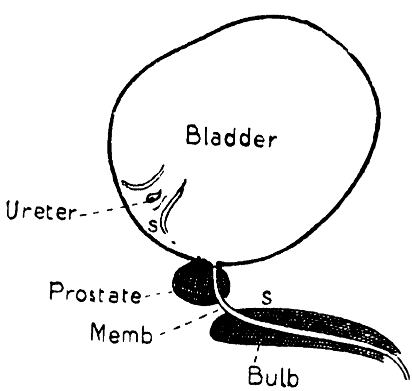


FIG. 599.

THE BLADDER is lined by transitional epithelium, closely adherent to the underlying muscular coat. In the latter are three layers of muscular fibres:

(1) An outer layer, running longitudinally from the neck of the bladder to the fundus, sometimes distinguished by the name of the *detrusor urinæ*. At the neck of the bladder these bundles send some fibres to be attached to the pubes as the pubo-vesical muscles. On the dorsal surface some bundles, in the male, pass on to the prostate and the urethra, while in the female they end in the tough connective tissue in the urethro-vaginal septum.

* VERNEY and VOGT. *Q. J. Exp. Physiol.*, 1938, **28**, 253.

(2) The middle layer, the thickest of the three, arranged circularly and forming a continuous layer.

(3) The inner layer thin and incomplete.

The name of *detrusor* ought physiologically to be applied to the whole of the three coats, which act as one in diminishing the capacity of the bladder.

THE SPHINCTERS. At the base of the bladder, the middle layer is modified over the trigonum by fibres, which serve as the most important sphincter, the *sphincter vesicæ*, or *internal sphincter*. Its fibres pass obliquely forwards and downwards and form a loop around the orifice of the bladder, lying on the ventral side of the bladder below and quite distinct from the thick coat of circular fibres belonging to the bladder itself (SS, Fig. 599; Fig. 600). If a catheter be passed into the urethra no urine escapes until

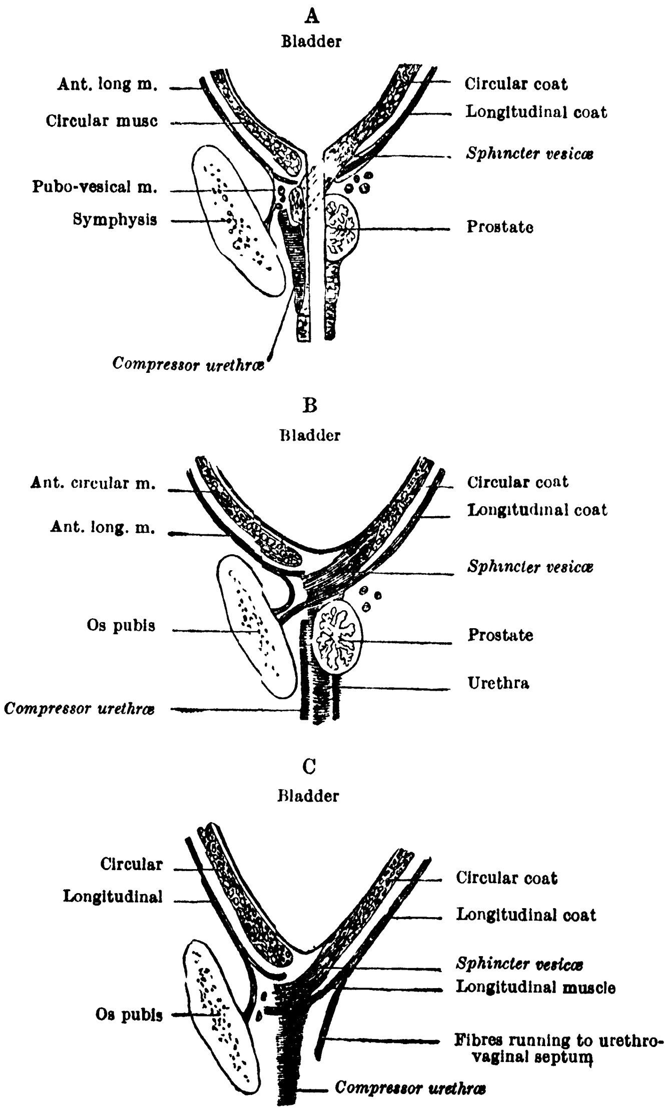


FIG. 600. Sagittal Sections through Neck of Bladder. (METZNER after KALISCHER.)

A. In middle line (male). B. Slightly to left of middle line (male). C. Ditto (female).

its orifice has passed the internal sphincter and actually entered the bladder. The sphincter muscle in the male is continuous with the muscular tissue of the prostate, and in the female with the muscle of the urethral wall; these continuations of the sphincter, by their tonic contraction, will also tend to prevent the escape of urine along the canal.

This urethral muscle is strengthened by two other sphincter muscles, which are composed of striated fibres. Together they form the *external sphincter*. They can be closed voluntarily, but are only opened reflexly and as part of the act of micturition. The chief one, the *compressor urethræ*, forms a flat ring around the second part of the urethra, extending in the male from the prostate to the bulb, where its function is taken up by the *bulbo-cavernosus* (Fig. 600).

The bladder is therefore supplied with a powerful muscular wall, the contraction of which will cause its evacuation, and with sphincters of two kinds, one involuntary, the internal *sphincter*, at the neck of the bladder, and two voluntary external sphincters, the *compressor urethræ* and *bulbo-cavernosus* muscles, which can empty the lower parts of the urethra. In man, after destruction of the first part of the urethra by suprapubic prostatectomy, the compressor urethræ is the competent sphincter.

The Nerve Supply of the Bladder and internal sphincter (Fig. 601) is derived from two main sources, namely, from the hypogastrics (sympathetic) and from the

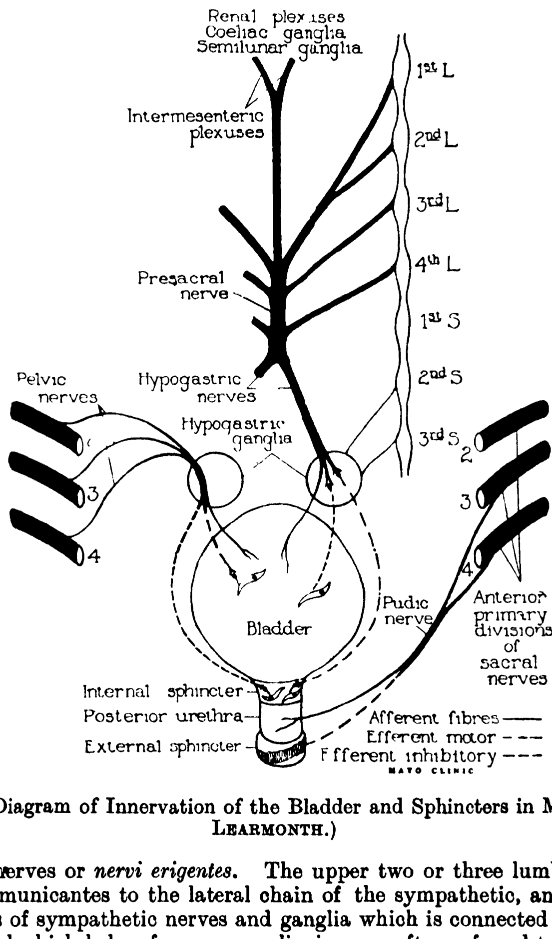


FIG. 601. Diagram of Innervation of the Bladder and Sphincters in Man. (After LEARMONTH.)

pelvic visceral nerves or *nervi erigentes*. The upper two or three lumbar nerves send white rami communicantes to the lateral chain of the sympathetic, and thence to the collateral plexus of sympathetic nerves and ganglia which is connected above with the aortic plexus and which below forms a ganglionic mass often referred to as "the intermesenteric plexus." Most of the fibres then pass, by the plexus of hypogastric nerves, into the pelvis on each side of the rectum and in man end by relaying in the hypogastric ganglia, at the base of the bladder. From thence, fibres pass to the bladder wall. In

the cat the hypogastric nerves are post ganglionic, with cell stations in the inferior mesenteric ganglia.

The pelvic visceral nerves are derived from the second and third sacral nerves. They pass directly to the vesical plexus and are carried with branches of this plexus to the neck of the bladder. The fibres do not run directly from the spinal cord to their ending in the bladder wall, but are interrupted by cells situated peripherally, partly in the vesical plexus, but chiefly in the walls of the bladder itself. Both sets of fibres also supply the rectum and the colon, and carry efferent impulses to the bladder. Afferent impulses from the bladder and urethra travel in both, but chiefly in the pelvic visceral nerves, and enter the spinal cord by the dorsal roots of the sacral nerves, particularly the second and third.

The afferent and efferent nerves of the urethra travel in the pudic nerves and in the hypogastrics.

The muscles of the external sphincter are supplied by the pudic nerve.

ACTION OF THE PELVIC VISCERAL NERVES. About a third of the fibres of the pelvic nerve are afferent. Excitation of the peripheral end of one pelvic visceral nerve causes a strong contraction of the same side of the bladder, sometimes extending also to a slight extent to the contralateral half.

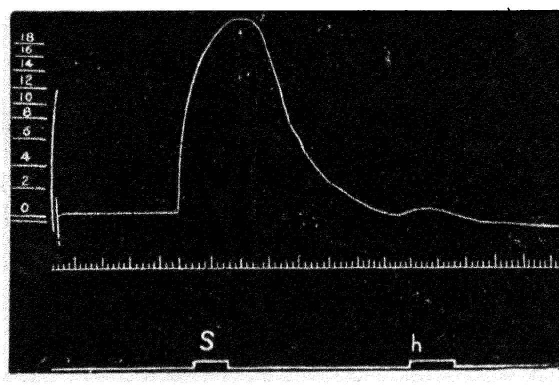


FIG. 602. Curve showing Rise of Pressure in the Bladder caused by Stimulation of S, Pelvic Visceral Nerves, *h*, Hypogastric Nerves. (FAGGE.)
The scale indicates centimetres of water.

When both pelvic nerves are stimulated simultaneously, contraction of both sides of the bladder causes a rise of pressure in its interior (Fig. 602) which is sufficient to overcome the resistance of the sphincter and to cause a complete emptying of the bladder.

Relaxation of the sphincter is also believed to be brought about by impulses descending the pelvic visceral nerves. There is no doubt, therefore, that these nerves are most important for the act of micturition.

Section of the nerve on one side causes no abnormality in micturition. Section of both pelvic nerves paralyses micturition, but power of partial evacuation of the bladder may return in a few weeks. If the hypogastrics be now cut, or even the sacral cord extirpated, the bladder is not completely paralysed, though its evacuation becomes unconscious and incomplete.

ACTION OF THE HYPOGASTRIC NERVES. These nerves show differences in their action, according to the animal and the conditions. In nearly all animals, including man, stimulation of the hypogastric nerves causes contraction of the trigone, ureteric openings, and internal sphincter. But in man, and many other animals, it may also excite either contraction or

relaxation (or both) of the detrusor, and so cause either a rise * or a fall of pressure in the bladder. Complete removal of the sympathetic supply to the bladder in man appears at first to result in some weakness of the internal sphincter, but this soon passes off and normal micturition is restored. Further, since stimulation may relax the detrusor in some cases, the retention of urine and the distention of the bladder may be aided by a reflex relaxation of the bladder wall and a reflex constriction of the sphincter, excited in each case through these nerves. We may therefore regard these nerves as normally and chiefly exercising a tonic effect on the internal sphincter, and micturition must be associated with a central inhibition of this tonic activity.

Normally, therefore, both sets of nerves are essential. The hypogastrics, by their undoubted action on the internal sphincter, play an especially active part during the accumulation of urine in the bladder, while the pelvic visceral nerves are necessary for the complete evacuation of the bladder which occurs at micturition.

Sensory fibres run in both nerves: those for pain of stretching of the bladder in the sympathetic, and those for touch, together with afferents involved in the micturition reflex, in the pelvics.

THE CONTRACTION OF THE BLADDER. The muscle of the bladder wall, being plain muscle, executes slow contractions. The nature and power of these vary according to whether the contractions are "isotonic" constant pressure in bladder) or "isometric" (bladder contents kept at constant volume).†

Under isotonic conditions, the normally innervated bladder exhibits regular rhythmic variations of volume, lasting some ten seconds, which increase in frequency and diminish in amplitude when the filling pressure is raised. Stimulation of the hypogastrics, or adrenaline in large doses, cause relaxation; stimulation of the pelvic nerves, or acetylcholine, cause contraction (but if the pressure maintained in the bladder is high, acetylcholine may cause relaxation).

Under isometric conditions, the bladder often remains quiescent for long periods, which alternate with powerful contractions, raising the pressure often to more than 100 mm. Hg, and lasting for a minute or more. These are abolished by section of the pelvic nerves and are probably reflex. Changing-over from the isotonic to the isometric state at the moment when an isotonic contraction is developing leads at once to a powerful isometric contraction, but if the change-over is made when an isotonic contraction is subsiding, a period of isometric rest at zero pressure commences.

It is probable that the initiation of powerful isometric contractions whenever the pressure in the bladder rises suddenly is a reflex from stretch receptors in the bladder wall. Afferent impulses along the pelvic nerves have been demonstrated when the bladder pressure is raised,‡ and once the reflex is started it is evidently self-exciting, and probably plays an important part in micturition.

THE FILLING OF THE BLADDER. Under normal circumstances, both sphincters are in a state of tonic contraction. Thus, it requires a considerably greater pressure in the bladder to overcome the resistance of the sphincters during life than after death. The urine, therefore, as it is

* MELLANBY and PRATT. *Proc. R. S.*, 1939, **127B.**, 307, 1940, **128B**, 186.

† J. P. EVANS. *J. Physiol.*, 1936, **86**, 396.

‡ McCREA and MACDONALD. *J. Physiol.*, 1936, **86**, 20P; LEARMONTH. *Brain*, 1931, **54**, 147.

secreted will normally accumulate in, and distend, the bladder. Let us first consider the filling of the normal bladder. The filling and emptying of the bladder in man can be studied by the introduction of appropriate catheters as shown in Fig. 603, the catheters being connected with optical recording manometers, shown in Fig. 604. The normal bladder with its proper innervation accommodates a good deal of fluid before showing any considerable rise of

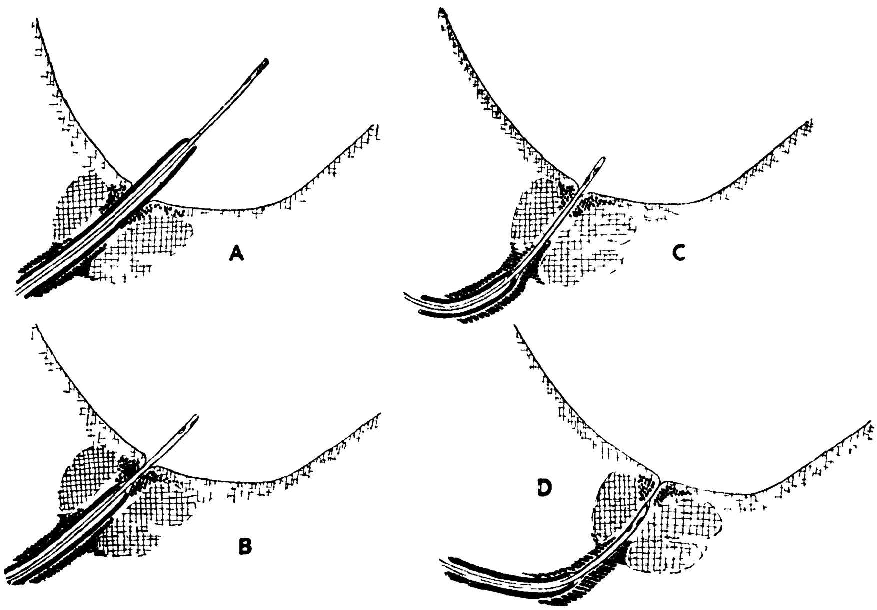


FIG. 603. Four arrangements of concentric double catheters for studying pressures in bladder, action of sphincters, etc. Internal sphincters stippled, external sphincter heavily hatched, prostate lightly cross-hatched. The wide catheter *a* is connected with the tube *f* (Fig. 604), and the narrow catheter *b* running inside it, with the bulb *l*. Fig A: both catheters in bladder, one for filling or emptying, one for recording pressure. Fig. B: only internal sphincter functional. If a leak is arranged in the outlet of the larger catheter, a rise of pressure in that catheter shows that the internal sphincter is relaxing and allowing fluid to leave the bladder. Similarly in position C and D, the relaxation of the external sphincter can be demonstrated. (DENNY-BROWN and ROBERTSON, *Brain*, 1933, **56**.)

pressure, or pronounced contractions. Even when fluid is introduced by a catheter 50 or 100 c.c. at a time, there is only a momentary tonic resistance, which soon subsides (Fig. 605). With a filling of about 400 c.c. the pressure rises more sharply, and at 600 to 800 c.c. there are powerful rhythmic contractions accompanied by painful sensations. By voluntary effort these can be suppressed for a time (Fig. 606), but when the pressure reaches about 100 cm. water micturition follows involuntarily.

The filling conditions are different in a bladder cut off from the central nervous system, as in patients some time after a lesion of the cauda equina and sacral cord. We may then regard the bladder as a tonic viscus with elastic walls containing plain muscle, and with a tonically contracted sphincter. If a distending force be applied rapidly, the sudden increase of tension acts as a direct excitant to the muscle, causing it to enter into contraction, which may be tonic or rhythmic.

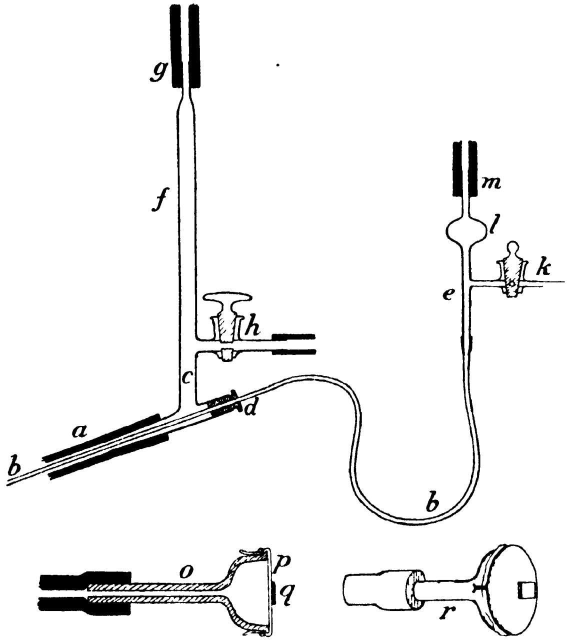


FIG. 604 (Top.) Filling and recording system for human bladder. Reservoirs of fluid connected and controlled through taps *h* and *k*.
(*Below.*) Optical manometers connected with *g* and *m*. *p*, rubber membrane; *q*, mirror. (DENNY-BROWN and ROBERTSON.)

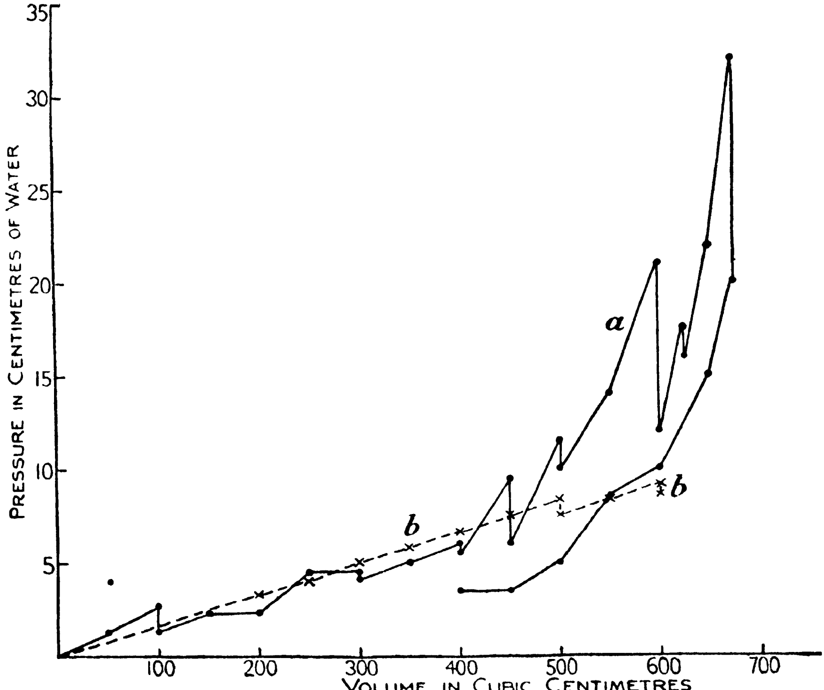


FIG. 605. *a*, Record of filling, followed by emptying, of normal bladder, in 50 c.c. steps. The vertical drops indicate adaptation during the intervals. *b*, Record of a filling just after bladder had been subjected to prolonged preliminary distension. (DENNY-BROWN and ROBERTSON.)

The effect of distension of the denervated bladder on the tension in this organ will depend, therefore, on the rapidity with which it occurs. In any

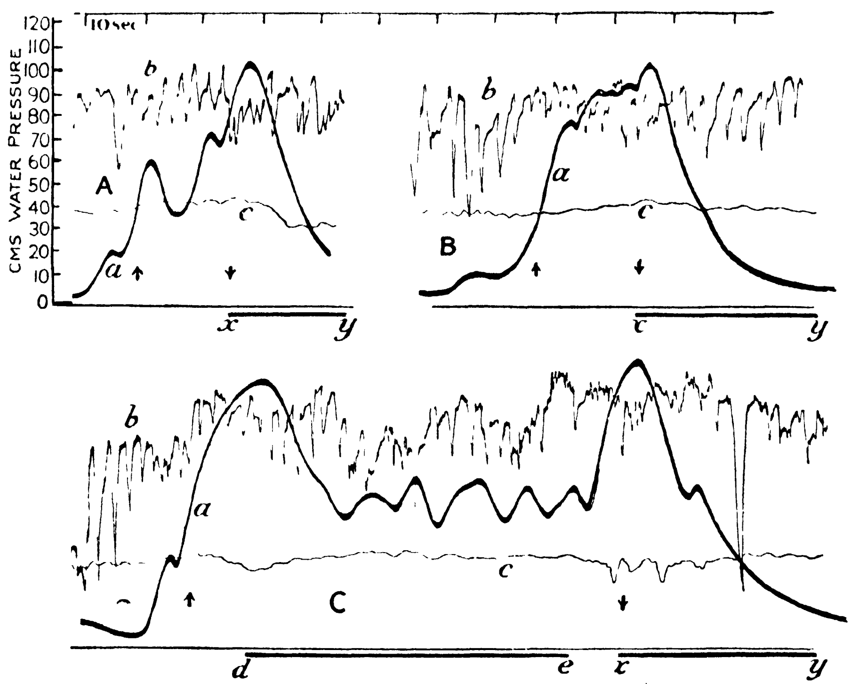
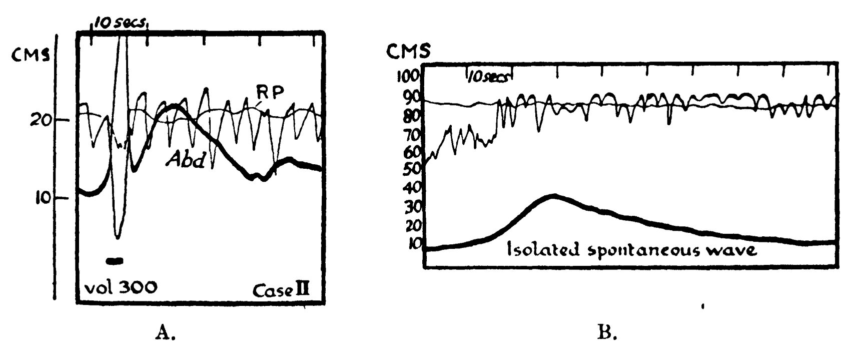


FIG. 606. Contraction Waves from Bladder of Normal Human Subject. *a*, vesical pressure (catheter in bladder); *b*, abdominal movements; *c*, rectal pressure. Arrows pointing up and down indicate opening and closing respectively of sphincters. $x - y$ = strong and $d - e$ weaker, efforts at restraint. (DENNY-BROWN and ROBERTSON.)

case, the denervated bladder is highly tonic and shows a considerable rise of pressure when fluid is injected into it by a catheter; thus 200 c.c. will give a pressure of 70 cm. water, which later may fall by slow adaptation to 30 cm.



A. B.

FIG. 607. Bladder Contractions from Patients with Lesions of the Cauda Equina. A, Simplest type of contraction: elicited by brief passive abdominal pressure. Duration of stimulus shown by short horizontal line. B, Lowest grade of compound wave: spontaneous. R.P., Rectal pressure. Abd., Abdominal movements. (DENNY-BROWN and ROBERTSON.)

or less. At such pressures the effect of the stretching of the muscular wall makes its appearance. A manometer connected with the bladder shows a series of rhythmic contractions of the muscular wall (Fig. 607, A), each lasting about a minute, and becoming more powerful as the distension of the bladder augments (Fig. 607, B). In the bladder entirely cut off from its connection with the central nervous system, these automatic rhythmic contractions gradually increase in force, until one of them suffices to overcome the resistance presented by the tonically contracted sphincter. A partial emptying of the bladder therefore takes place, but before the bladder has been emptied, the pressure falls below that necessary to overcome the resistance of the sphincter, so that there is always, under these circumstances, a certain amount of residual urine left in the bladder. This is the condition found in animals when the lower part of the spinal cord has been extirpated, or in man when it has been destroyed as the result of accident or disease.

MICTURITION. In the denervated bladder, the factor finally causing partial evacuation is the gradual increase in the intravesical tension, from the accumulation of fluid. The same factor is prepotent in determining the onset of micturition at lower pressures in the normal animal. The desire to micturate generally arises in normal adults when about 400 c.c. of urine has collected in the bladder ; it can be voluntarily overruled until the amount is much greater, but at about 700 c.c. becomes urgent and painful. The main path for these sensations is by the sympathetic, and the act of micturition is a reflex which is liberated from voluntary suppression. As a preliminary to this release the perineal muscles are relaxed. In the absence of restraint from the higher centres (*e.g.* in the infant), micturition will take place each time that the tension in the bladder has reached a certain height, the amount of fluid in the bladder at the time depending, on the one hand, on the rate at which the fluid has entered this organ from the ureters, on the other hand, on the irritability of the bladder wall itself and of the nervous centres concerned with its motor innervation. The effect of the gradual accumulation of fluid and rise of tension is twofold. In the first place, it acts on the bladder wall, causing rhythmic contractions of ever-increasing intensity ; in the second place, the mere stretching of the bladder originates impulses in the sensory nerve-endings in its wall, and these, as we have seen with the "isometric" bladder, are reinforced at every rise of tension caused by the rhythmic contractions. These impulses travel up to the nervous centres, and are summated until, when released, they result in a sudden discharge of efferent impulses of two kinds, namely :

(1) Motor impulses to the whole musculature of the fundus of the bladder.

(2) Inhibition of the tonic contraction of the sphincter muscles. The resultant of these two processes, the contraction of the detrusor and the relaxation of the sphincters, is a complete emptying of the bladder, and the act is completed by the contraction of the involuntary and voluntary muscles surrounding the urethra and causing complete expulsion of the contents of this tube.

Observations on man show that an active relaxation of the internal sphincter is a necessary part of the act of micturition. Thus a rigid catheter was introduced into the bladder, which was fully distended with fluid. On withdrawing the catheter until its opening lay just outside the internal sphincter, the flow of urine stopped. The man, however, was able to micturate directly he was told, and to stop again, though less suddenly, at will. It was impossible in this case for any of the urethral muscles to be concerned, since the rigid catheter impeded their action.

The Reflexes of Micturition. The investigations of Barrington * have revealed six closely co-ordinated reflexes concerned in micturition in the cat, viz. :—

(1) *Contraction of the bladder* when its internal pressure approaches 10 cm. of water. The afferent and efferent paths are in the pelvic nerves.

(2) *Contraction of the bladder*, even when only partially full, as a result of running fluid through the urethra. This ensures that when micturition is started by the first reflex, it will normally be continued to completion. The afferent path is the pudic, the efferent the pelvic nerve.

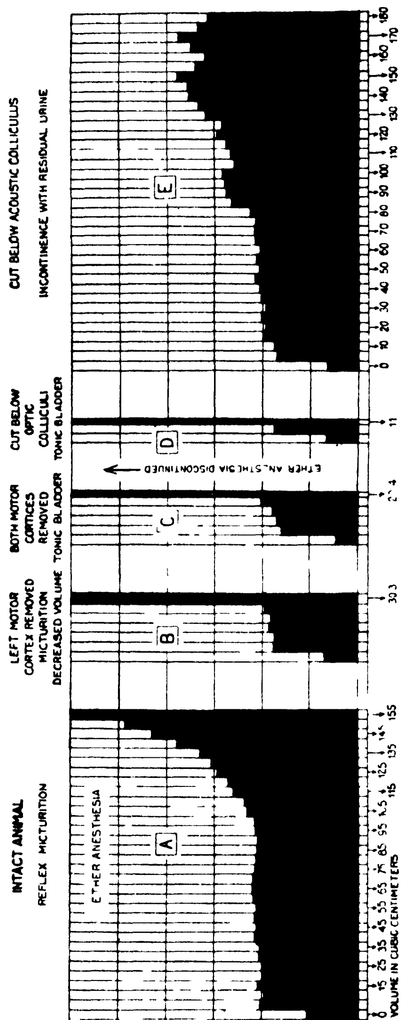


FIG 608. Effect of brain sections on volume-pressure relations and emptying of bladder in etherised cat. (LANGWORTHY and KOLB, *Brain*, 1933, **56**, 371.)

(3) *Contraction of the bladder* (slight), when the first part of the urethra is distended. Both paths are in the hypogastrics.

(4) *Relaxation of the urethra* when fluid passes along it. Both afferent and efferent paths are in the pudic nerves.

(5) *Relaxation of the urethra* when the bladder contracts. The afferent path is in the pelvic nerves, the efferent in the pudic.

(6) *Relaxation of plain muscle of proximal third of the urethra*, when the bladder is distended. Both paths are in the pelvic nerves.

It will be seen that each of these reflexes, except the third, is capable of giving rise to the remainder, and so collaborating to ensure normal micturition. There is no doubt that similar reflexes are operative in man.

THE CENTRAL CONTROL OF THE BLADDER. Like many complex reflexes, micturition is under the joint control of higher and subsidiary nerve centres ; in all there are at least three levels of control—namely, cortical, brain-stem and spinal. Control from the hypothalamus has also been claimed. The nature of this control is illustrated in Fig. 608, which shows the progressive rise of bladder pressure in the cat as the contents are incremented in 5 c.c. steps, until finally evacuation occurs, complete or otherwise. With the intact animal the reflex occurred at 155 c.c. ; after removal of one or both cerebral cortices the reflex was much more easily started and relaxation to

* BARRINGTON. *Q. J. Exp. Phys.*, 1914, **8**, 33 ; 1915, **9**, 261 ; 1925, **15**, 81 ; *Brain*, 1921, **44**, 23 ; 1922, **45**, 126 ; 1928, **51**, 209 ; 1931, **54**, 177.

accommodate the contents was slight. Section below the superior colliculi led to so excitable a reflex that evacuation took place at only 11 c.c., but section below the inferior colliculi gave an atonic bladder, showing retention with overflow, *i.e.* with incomplete emptying and no proper reflex.

In the human adult, the processes of retention and evacuation of urine are modified and controlled by reflexes and by voluntary effort. The human bladder shows a readier accommodation to increase of its contents without undue rise of pressure when its nerve supply is intact than when it has been denervated, even though connections with higher centres have been cut off as, for instance, after section of the cord in the mid-dorsal region. But its capacity is still further improved by the influence of the higher centres, which can exercise considerable restraint over the reflex micturition mediated by the lower centres. The normal action of the sphincter mechanism may be aided by the contraction of the perineal muscles, which keep the urethra closed. Under usual conditions, however, the intact subject micturates before much conscious restraint has had to be exercised. In normal micturition there is first of all a voluntary removal of restraint, so releasing the reflexes of micturition which are effected by the lower centres. These reflexes involve the contraction of the bladder and the release of the sphincters. If the contents of the bladder are small in volume, the reflex process of evacuation may be set in motion by voluntary contraction of the abdominal muscles, by which the pressure in the bladder is increased and the sphincter action reflexly inhibited (reflexes 5 and 6 above).

The third, fourth, fifth and sixth of the reflexes described by Barrington are spinal, the centre concerned being situated in the lumbo-sacral spinal cord. If this centre and its connections be intact, reflex micturition may be carried out even after section of the cord in the dorsal region, but according to Barrington, the process of micturition is always imperfect in animals and in man after section of the cord in any part of its course. In Barrington's first and second reflexes the dominant centre in the cat is in the hind-brain, and when the paths connecting the subordinate centre in the lumbo-sacral cord, with the hind-brain are divided, or the higher centres injured, the emptying of the bladder remains incomplete. The spinal centre can be excited reflexly by stimulation of any sensory nerve, such as the sciatic or the fifth nerve. In many cases where, in consequence of obstruction to the passage of impulses from the higher parts of the central nervous system, micturition is delayed, this act may be excited by the application of cold or hot sponges to the perineum, and it is well known that irritation of the pelvic organs in children may give rise to reflex involuntary micturition.

Barrington finds in cats that normal micturition is lost after destruction of a small area of the midbrain situated a short distance ventral to the internal edge of the superior cerebellar peduncle, and extending from the level of the posterior end of the aqueduct in front to the middle of the motor nucleus of the fifth nerve behind.

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CHAPTER XLVIII

THE SKIN AND THE SKIN GLANDS

In all classes of animals, the skin performs two functions. In the first place, it serves to protect the underlying parts from injury and from invasion by foreign organisms. In the second place, it serves as a sense organ, and is richly supplied with nerves, by means of which the activities of the body as a whole may be brought into relation with the changes going on in the

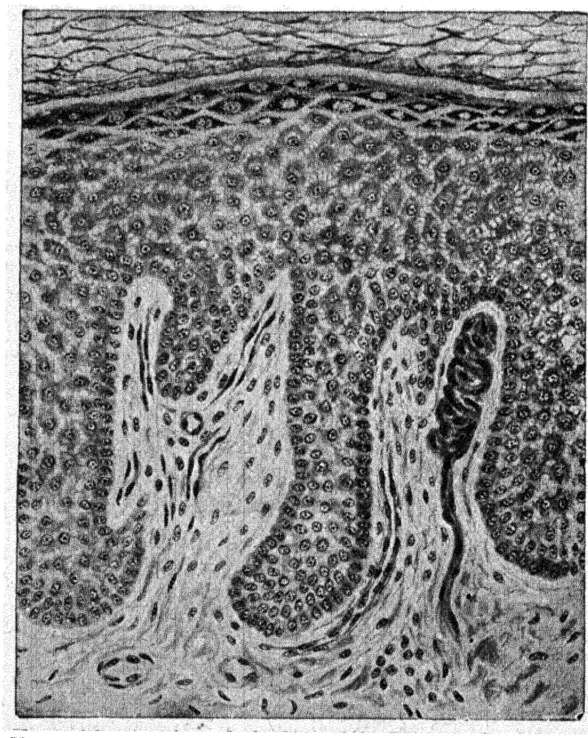

FIG. 609. Vertical Section through the Skin of the Palmar side of the Finger, showing two Papillæ (one of which contains a Tactile Corpuscle) and the deeper Layers of the Epidermis. Magnified about 200 diameters. (SCHAFER.)

environment. In warm-blooded animals, the skin plays an important part in the regulation of the body temperature, since the loss of heat from the body must occur almost entirely through its surface. It also has important functions as a place for the storage of certain materials.

The development of the skin as an organ of protection shows wide modification in various classes of animals. Thus it may become the seat of formation of horny plates, as in the alligator; of poisonous glands, as in the toad; or of mucous glands, as in many varieties of fishes. In warm-blooded animals, the development of hair from the deeper layers of the epidermis serves to diminish the loss of heat. Since the hair follicles are

richly supplied with nerve fibres, the hairs act also as organs of sensation. In man, where the hairs are rudimentary, except in certain localities, practically only this tactile function is retained. The external layer of the skin in man consists of a tough horny layer formed by the keratinisation of the external layers of cells of the epidermis. The skin is composed of two parts, the epidermis and the cutis (Fig. 609). The epidermis is a stratified squamous epithelium. The deeper layers form the *rete mucosum*, being soft and protoplasmic, while the superficial layers forming the cuticle are hard and horny.

The deepest layers of the *rete mucosum* often contain granules of a pigment, *melanin*; in the coloured races this is abundant, and it is also produced in white skins as a result of sunburn. The formation of melanin is due to the presence of an oxidase in the *melanoblast* cells; fresh or formalised sections of skin rapidly form melanin in these cells if immersed in a solution of 3 : 4 dihydroxy-phenylalanine.* This substance is the first stage in the oxidation of tyrosine by the oxidase called tyrosinase,† and we may suppose that tyrosine is the parent substance of melanin.

The most superficial layer of the rete mucosum is formed of flattened cells filled with granules of a material staining deeply with hæmotoxylin and eosin, known as *eleidin*. This layer is called the *stratum granulosum*. Immediately superficial to this layer is another in which the cells are indistinct. The cells are clear in section and form what is known as the *stratum lucidum*. These two layers evidently constitute the intermediate stages in the transformation of the cells of the rete mucosum into the horny scales which make up the *stratum corneum*.

The cutis, or corium, is composed of dense connective tissue, which becomes more open in texture in its deeper part, where it merges into the subcutaneous connective tissue. The elasticity of the skin is due to the presence in the corium and subcutaneous tissue of numerous fine elastic tissue fibres. In old age this is diminished in amount, and the white fibres increased, especially where wrinkles occur. The most superficial layer of the corium is prolonged into minute papillæ, over which the epidermis is moulded. These papillæ contain, for the most part, capillary blood vessels; a few contain touch corpuscles, special organs of tactile sensation. The blood vessels of the skin form a close capillary network immediately at the surface of the cutis, sending up loops into the papillæ. Direct anastomoses between arterioles and venules (arterio-venous anastomoses) are common in the skin in parts liable to become cold, *e.g.* the hands, feet and digits in man, the ear in rabbits, &c.‡

All parts of the skin, except the palms of the hands and the soles of the feet, are beset with hair follicles. The hair follicles are small pits which extend downwards into the deeper part of the corium, being downgrowths of the rete mucosum. The hair growths form a small papilla of cells at the bottom of the follicle, the part of the hair lying within the follicle being known as the hair root. The hair itself consists of long tapering, horny cells, the nuclei of which are still visible, though the cell substance has been almost entirely converted into keratin.

THE SEBUM. The cuticle is kept supple and preserved from the drying effects of the atmosphere by being constantly impregnated with a fatty material known as *sebum*. This material is formed by the *sebaceous glands*, which are distributed all over the surface of the skin wherever hair follicles are to be found, the mouths of the glands opening into the hair follicles. A sebaceous gland is a pear-shaped body, consisting of a secreting part and a short neck opening into the follicle. The gland proper is composed of a solid mass of cells. The outermost cells are flattened and generally show signs of proliferation. The cells lying internal to these are much larger, and their protoplasm is transformed into a network, in the meshes of which are granules which show the reactions of fat. Further inwards, the protoplasmic network diminishes in amount, while the fatty granules increase in size, so that, in the lumen adjoining the duct, we find only a mass of cell débris and masses of fatty material. It has often been thought that the secretion of sebum depended simply on a fatty degeneration of the cells. The granules, however, when they first appear, stain with acid fuchsin, but not with osmic acid, and one must regard the formation of sebum as an act of true secretion, in which the secretory granules are gradually transformed into the special constituents of the sebum. The fatty acids of the sebum are for the most part in combination, not with glycerol but with higher alcohols, including isocholesterol. Wool-fat or lanoline, which may be extracted from wool, as well as from the feather-glands of water birds, such as the goose

* BLOCK. *Z f. physiol. Chem.*, 1917, **98**, 226.
† RAPER. *Physiol. Rev*, 1928, **8**, 245.
‡ GRANT. *Heart*, 1931, **15**, 281, 385.

and duck, consists mainly of esters of isocholesterol. It presents many advantages over ordinary fat as a protective salve for the surface of the body. In the first place, it can take up a large amount, as much as 100 per cent., of water. In the second place, it is not attacked by micro-organisms, so that it does not tend to become rancid.

The secretion of sebum is a continuous process, though it is probably quickened in conditions of increased vascularity of the skin. The extrusion of the products of secretion is determined by the presence of unstriated muscle fibres, the *arrectores pilorum*, which pass from the surface of the cutis obliquely over the outer surface of the sebaceous gland. They are supplied by sympathetic nerves. When these muscle fibres contract, the hair is erected and a certain amount of the sebum squeezed out on to the root of the hair and the surrounding skin. This contraction will occur on faradic stimulation, or whenever cold is suddenly applied to the skin. In some animals, including man, contraction also occurs in response to adrenaline.* The contracted condition of all the muscles of the hair follicles is shown by the 'goose-skin' produced under such circumstances. There is no evidence that the secretion of sebum is in any way under the control of the central nervous system.

THE SWEAT GLANDS. The sweat glands are distributed over the whole surface of the skin, and are especially abundant on the palm of the hand and on the sole of the foot. They are composed of single, unbranched, coiled tubes, which lie in the subcutaneous tissue and send their ducts up through the cutis, to open on the surface by corkscrew-like channels which pierce the epidermis. The secreting part of the tube consists of a basement membrane lined by a double layer of cells; the innermost of these are cubical and represent the secreting cells proper. Between the secreting cells and the basement membrane is a layer of unstriated muscle fibres. The duct of the gland has an epithelium, consisting of two or three layers of cells with a well-marked internal cuticular lining, but there is no muscular layer.

In addition to these, which are sometimes called the *eccrine* sweat glands, there are in certain situations, *e.g.* axilla, larger glands somewhat resembling sebaceous glands, and often, like them, associated with hairs. They are called *apocrine* glands and secrete a fluid containing a fatty material of peculiar odour.

Insensible Perspiration. Under normal circumstances and at rest, in temperate climates the greater part of the water taken in with the food is excreted by the kidneys, a smaller proportion leaving by the lungs and by the surface of the skin. The last two are, in states of rest, about equal, and vary with the metabolism of the individual and with the temperature and humidity of the air. Benedict and his colleagues † estimate the loss at about 18 grams per hour for each, in a man with a basal metabolism of 1,700 cals.: this would amount to about 430 grams from the skin and a like amount from the lungs in the twenty-four hours. In the more humid atmosphere of Europe it may be less. These estimations refer, of course, to the condition in which there is no visible sweating, but only a loss of so-called "insensible perspiration" which is continually going on. It is apparently not due to slight sweating, but to a passage of water vapour by osmosis, through the skin,‡ and, as it seems, not by way of the sweat glands at all.§

Sweating. Many sweat glands are inactive and incapable of secreting, but there are from 2 to 3½ million functional sweat glands in the body.||

* Elliott. *J. Physiol.*, 1904, **32**, 416.
† Benedict and Root. *Arch. Inst. Med.*, 1926, **38**, 1.
‡ Hancock, Whitehouse and Haldane. *Proc. Roy. Soc.*, 1930, **105B**, 43.
§ Vasti. *Amer. J. Physiol.*, **1932**, **102**. 60.
|| Kuno. *Lancet* 1938, **234**, 299.

The excretion of sweat by the skin is mainly determined by the need for regulating the temperature of the body, so that the water lost in this way depends on the heat production of the body or on the external temperature, and is very little affected by alterations in the quantity of fluid drunk. If a man's body be enclosed in a vessel through which a current of air is passed, and the temperature of the air gradually raised, it will be noted that the amount of water given off rises slowly up to a certain degree and then mounts rapidly. The sudden kink in the curve is due to the commencement of the activity of the sweat glands (Fig. 610), *i.e.* to onset of sweating.

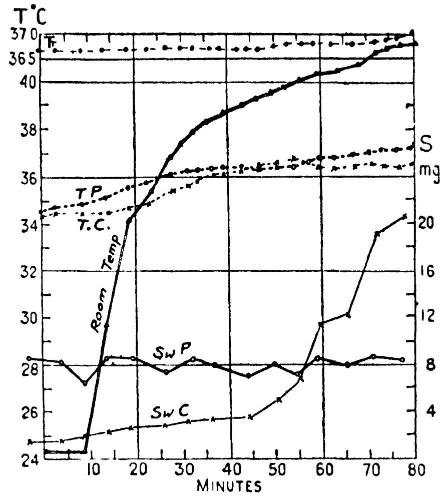

Fig. 610. Sweating in m.g. per 20 sq. cm. per 5 minutes from palm (sw.p.) and chest (sw.c.) with rise of room temperature. Tr. T.P., T.C. = temperatures in rectum, on palm and on chest respectively. (Kuno, *Lancet*, 1930.)

The sweat, which mainly consists of the secretion of the eccrine glands, is the most dilute of all animal fluids. As collected, it generally contains epithelial scales and some sebum, and is usually acid in reaction; the pH, however, may range from 4 to 8, according to the extent to which the lactic acid is neutralised. The composition of sweat varies considerably, but the following results are typical:—

Composition of Human Sweat.*

Water		99·742–99·221 per cent.
Solids		0·258–0·779 ,,
Organic solids		0·03–0·290 ,,
Ash		0·144–0566 ,,
Chlorine (usually over 0·15 per cent.)		0·059–0·346 ,,
Potassium	about	0 017 per cent.
Sulphate	,,	0·004 ,,
Lactic acid	,,	0 070 ,,
Urea	,,	0·03 ,,
Sodium	,,	0·15 ,,
Sugar	,,	0·004 ,,

The only plentiful constituent is sodium chloride, the amount of which varies, though sweat is always hypotonic. In profuse sweating a man may lose 10 g. or more of Cl in three hours. In winter, or in subjects unaccustomed to sweating, the chloride content is much higher (*e.g.* 50 mE. per l.) than in those acclimatised to heat or used to sweating (*e.g.*, 25 mE. per l.). The more rapid the sweating the higher the chloride concentration. Urea is present in about the same amount as in blood, but lactate seems to be either concentrated or formed by the sweat glands. After filtration, sweat forms a clear colourless fluid of a specific gravity of about 1003.

The secretion is under the control of the central nervous system and, except for that of the palms and soles, has been shown by Kuno † to be

* Based on Whitehouse. *Proc. Roy. Soc.*, 1935, **117B**, 139.

† Kuno. "The Physiology of Human Perspiration." (Churchill, 1934.)

almost entirely adapted to the regulation of the body temperature. The nervous mechanism can be set into activity either centrally or reflexly. The most usual cause is a rise of the body temperature. If a man sits in a warm room, *e.g.* of a Turkish bath, the secretion of sweat commences as soon as the temperature of the body has attained a height of 0·2° to 0·5° C. above normal. It has been shown by Kuno and his associates that sweating is not uniform in different parts of the body. The palms and soles do not react to the rise of temperature, and the axillæ and internal femoral regions sweat little, while the head, neck and dorsal and ventral (but not lateral) surfaces of the upper trunk sweat most. It will be seen that most sweating occurs in those regions which, by reason of their exposure, offer the best opportunities for evaporation.

The palms and soles are peculiar inasmuch as they constantly secrete some sweat, but the amount is not increased by rise of temperature (Fig. 610). It does, however, show an increase under direct nervous control, as in exercise, anxiety, or mental stress of any kind (Fig. 611). This probably applies also to the pads of the cat, which cannot play an important part in the regulation of body temperature, since they are the only parts of its body provided with sweat glands.

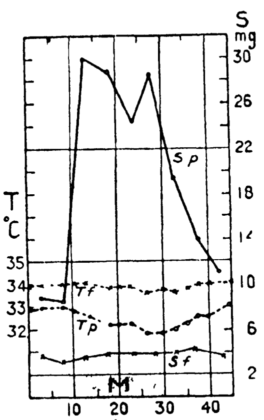

FIG. 611. Sweating of palm (s.p.) and forehead (s.f.) and temperatures of palm and forehead. Mental arithmetic during M. (KOSAKA. *J. Oriental Med.*, 1929, **10**, 75.)

Sweating is facilitated by muscular exertion, and the temperature will generally be found to be raised if it be taken at the instant that sweating has commenced. The effect of rise of temperature may, however, be partly local, so that one arm enclosed in a hot-air bath may sweat more than the rest of the body. Under ordinary circumstances, the central stimulation by the warm blood is the predominant factor. Sweating may be induced in the cat's paws by warming the blood passing through the carotid arteries on its way to the brain, at a time when the temperature of the blood circulating through the rest of the body, including the paws themselves, has undergone no alteration. Sweating may also be aroused by asphyxia, and this result is found even in the spinal cat, *i.e.* after separation of the spinal centres from the medulla. The secretion of sweat, although generally associated with increased vascularity of the skin, is not entirely dependent thereon. Thus, even in the amputated limb, stimulation of the sciatic nerve may cause the appearance of drops of sweat on the pad of the foot. If the sciatic nerve be stimulated in the intact animal, the secretion of sweat which is produced is associated with constriction of the vessels of the skin, due to simultaneous stimulation of the vasoconstricton fibres running in the sciatic nerve.

As Langley showed,* the sweat nerves are sympathetic. Leaving the cord by the white rami communicantes from the second dorsal to the third or fourth lumbar nerves, they pass into the sympathetic chain. Here the first relay of fibres ends in connection with the cells of the sympathetic ganglia, and a fresh relay of fibres, which are non-medullated, pass from the cells along the grey rami into the various spinal nerves, to be distributed to the whole surface of the skin. There are certain anomalies connected with sweat nerves, however. Thus adrenaline has, in man, no action, whereas

* LANGLEY. *J. Physiol.*, 1891, **12**, 347.

pilocarpine, though regarded as a parasympathetic stimulant, causes abundant sweating, even after division and degeneration of the sweat nerves, so that this drug must act peripherally on the glands. Complete section and degeneration of all the nerves to a limb, however, greatly reduces the response to pilocarpine, because it also reduces the vasodilator response (Burn).* The action of pilocarpine, as well as the effects of artificial stimulation of the sweat nerves, is abolished by the administration of atropine. We may therefore suppose that, as in the case of the sympathetic ganglion synapse, the "chemical transmitter" in the case of the sweat glands, is not adrenaline, but acetyl choline, which substance does, in fact, cause sweating. The injection of pilocarpine, or pituitrin into the cerebral ventricles also excites profuse sweating,† apparently by exciting the diencephalic centres connected with the origins of both sympathetic and parasympathetic systems.

Absorption by the Skin. In order to test the alleged influence of baths containing medicinal substances in solution, many experiments have been made to determine whether absorption is possible by the skin. It may be regarded as established that the uninjured skin is impermeable to watery solutions of salts or other substances. On the other hand, it is possible to produce a certain amount of absorption by the application of substances dissolved in fatty vehicles. Thus, the administration of mercury is often carried out by the inunction of mercurial ointments, and the fact that mercurial salivation may be produced in these conditions shows that a certain amount of the mercury must have been absorbed. It is difficult to imagine that any appreciable amount of cod liver oil will be available for the nutrition of the infant when this substance is administered by rubbing it on the skin. On the other hand, the moist mucous surfaces, such as the conjunctiva or the mucous membrane of the respiratory passages, as well as raw surfaces of the skin, *e.g.* which have been deprived of their epidermal layer by the application of blisters, permit the rapid passage of substances in watery or oily solution.

GASEOUS EXCHANGES THROUGH THE SKIN. In any animal with a thin moist skin, such as the frog, the absorption of oxygen and the excretion of CO_2 through the skin may be sufficient for the proper aeration of its blood, so that it may continue to live after the extirpation of its lungs. In man there is also a continuous output of CO_2 through the skin, but the amount leaving the body in this way is negligible compared with that which is exhaled through the lungs. The loss of CO_2 by the skin rises with increase of external temperature. Thus at a temperature of 29° to 33° C. the CO_2 output by the skin is about 0·35 g. per hour, *i.e.* about 8·4 g. in the twenty-four hours. When the external temperature rises above 33° C., the CO_2 output increases, so that at 34° it is doubled and at 38·5° it may amount to as much as 1·2 g. per hour (Schierbeck). It is just at this temperature of 33° C. that a secretion of sweat begins to be noticeable, so that it seems probable that the increased CO_2 output may be due to the secretion of sweat, which, like other body fluids, contains carbon dioxide that readily escapes from it on exposure to the air.

STORAGE FUNCTIONS OF THE SKIN. The corium, together with the subcutaneous tissue of the skin, has considerable capacity for storing various materials, provisionally or permanently. Of these the best known is fat, which is laid down in fat cells as a permanent store of subcutaneous adipose tissue.

The other type of storage which takes place in the skin is what has been called by Cannon *storage by inundation*, and which may be compared with the storage of water in a bog. The areolar tissue of the skin, and in other situations, has a marked affinity for water, and is an important place for its storage. Excess water retained in the body is held most conspicuously in the subcutaneous tissues, and, in pathological states, may lead to œdema; conversely, in water deprivation the subcutaneous water stores are freely drawn upon. After severe hæmorrhage, this may happen very quickly, about half the water leaving the tissues to enter the blood coming from the skin, the remainder chiefly from the muscles.

When the blood is flooded with soluble substances, these may likewise find temporary storage, accompanied by water or not, in the subcutaneous tissues. The most note-

* BURN. *Journ. Physiol.*, 1922, **56**, 232; 1925, **60**, 365.

† CUSHING. Papers relating to the pituitary body, hypothalamus and parasympathetic nervous system. (Ballière, Tindall and Cox, 1932.)

worthy instances are of glucose and salts. When the blood sugar level rises suddenly, considerable amounts of sugar may find temporary lodgment in the subcutaneous tissues, and as the heavy load of sugar is removed from the blood for utilization, or storage as glycogen, so the flood of sugar subsides from the connective tissues into the blood again. It is similar with chlorides. When a chloride-rich diet is given, or after intravenous chloride infusion, the heaviest storage of chloride is in the skin, while in chloride starvation, in which the blood chloride is preserved almost unchanged, as much as 90 per cent. of the chloride lost from the body may have come from the skin, though this tissue forms only 16 per cent. of the body weight.

THE HAIR

Hair in the lower animals has definite functions in relation to heat regulation, but in man this is of slight importance. The beard is a male secondary sexual character, as is hair on the chest and abdomen, and is not developed in men castrated before puberty. The hair of the axillæ and pubic regions is a secondary character of both sexes and is undeveloped after early removal of the gonads. There are considerable racial variations in hair.

There is no doubt that the growth and distribution of hair follicles is affected by hormones and vitamins, but exact knowledge of the effects is not yet available. The suprarenals seem to exert an influence, as in suprarenal tumours hair growth may be abnormal, but since sexual precocity is also a feature, an influence of the gonads may be more fundamental. The thyroid hormone is also essential to the proper growth and normality of the hair.

Curliness or straightness has not been satisfactorily explained; the former belief that hairs curled because they were elliptical in cross section instead of circular appears to be inadequate to explain it. Artificial curling, as in "permanent waving," is due to elongation of some of the filamentous keratin molecules by stretching under heat (*vide* p. 49).

Pigmentation, due to deposition of melanin, may be diffuse, as in red hair, or granular, as in black. The pigment tends to bleach in sunlight.

Hairs do not grow indefinitely, but after a period of growth the length remains stationary and the hair sooner or later may fall, or be pulled, out, after which a fresh cycle begins. Each hair follicle has its own characteristic growth rate and cycle; on the average the head hair grows about 0·4 mm. daily and normally reaches 65 cm. in length if uncut, *i.e.* growth continues for about four years. Short hairs such as those on the forehead, grow more slowly. Cutting or shaving off the hair has not been proved to modify the rate of growth.

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CHAPTER XLIX

THE TEMPERATURE OF THE BODY AND ITS REGULATION

In the chemical changes in the body the sum of the metabolic processes is associated with the evolution of heat. In man, under normal circumstances, while doing moderate work, the total daily energy requirements amount to about 3000 Calories, the whole of which is derived from the oxidation of the food. Of this energy, only about one-twentieth leaves the body as mechanical energy, the rest being evolved in the form of heat and being expended in the maintenance of the body temperature, or in the warming of the surrounding medium.

The evolution of heat is common to all living beings. It is very evident, for instance, in the germination of seeds. The interior of a bee-hive has a temperature often 10° above that of the surrounding atmosphere. Whenever we can excite increased activity in an organ, we are able to show that such activity is associated with increased evolution of heat. Since all chemical processes are quickened by rise of temperature, we should expect to find that the heat produced in the metabolic processes of organisms would tend in itself to quicken these processes. In most chemical reactions a rise of about 10° C. would increase the velocity of reaction from two to three times, and the same rule is found to hold approximately over short ranges of temperatures for *isolated* living tissues. The diagram (Fig. 612) shows the influence of temperature on the chemical changes in plain muscle, as measured by the rate of oxygen usage. The increase in oxygen intake by the tissue is increased about two and half times for a rise of 10° C. until about 40° C., beyond which temperature it declines

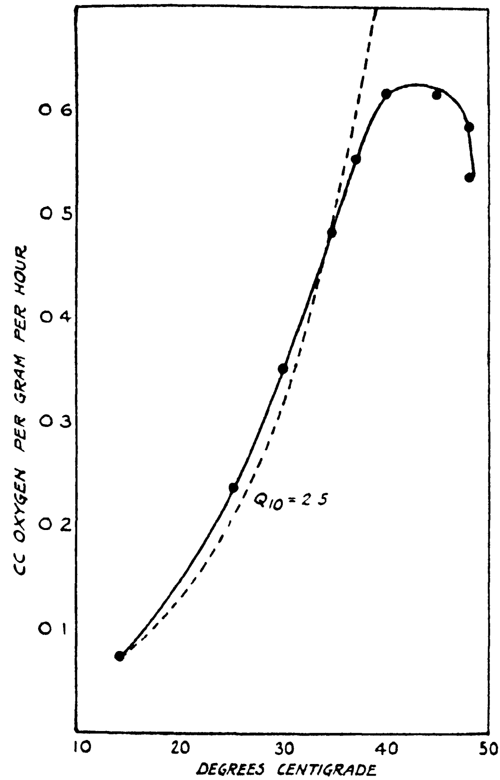

Fig. 612. Effect of Alteration of Temperature on Oxygen Usage of Plain Muscle (Gunea-pig Uterus). Abscissæ = temperature. Ordinates = c.c. oxygen per gram per hour. The dotted line represents the theoretical curve if the oxygen intake were increased two and half times for a rise of 10° C.

rapidly owing to damage to the tissue. At about 48° the tissue becomes inexcitable (heat paralysis), but will recover again if cooled.

In the animal organism, at rest and under standard conditions, we shall also expect to find that the rate of the metabolism is proportional *to the temperature of the animal.* This is universally the case, whether we are dealing with warm-blooded or cold-blooded animals. In warm-blooded animals the body temperature normally varies but little with changes in the temperature of the environment, whereas in cold-blooded animals, the temperature of the

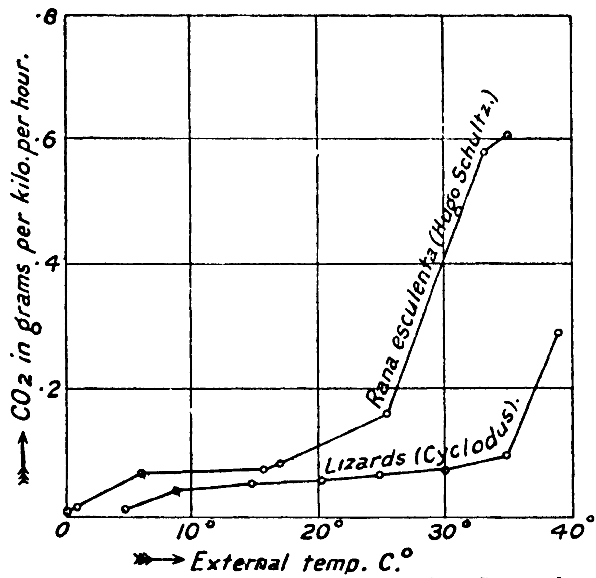

FIG. 613. Effect of Alterations in the Temperature of the Surrounding Medium on Output of CO_2 in Cold-blooded (Poikilothermic) Animals. (C. J. MARTIN.)

body, and therefore the amount of its heat production, is proportional to the external temperature (Fig. 613).*

It might be thought that such a reaction to change of temperature in cold-blooded animals would result in a vicious circle. Since the animal is continually producing heat, and thus raising its temperature above that of its surroundings, one might expect to find that the higher the external temperature the more would the temperature of the animal exceed it, until finally the latter would rise to such a height that the animal would die of heat-stroke, its tissues being damaged by the temperature ultimately attained. A certain protection is afforded to most cold-blooded terrestrial animals by the fact that their surface is moist, and that, with a rise of external temperature, the rate of evaporation from the surface increases, so that the quickened rate of cooling by evaporation may more than correspond to the rate of increase in the heat production, which would tend to raise the body temperature. The extreme variability in the metabolism of such animals implies a state of dependence of all the activities of the body on the environment, which would prevent the utilisation to the full of the available sources of energy.

An animal whose metabolism was more or less independent of the surrounding temperature must have a great advantage over an animal liable to

* MARTIN. *Phil. Trans. Roy Soc.*, 1903, B. 195, 1.

have his activities reduced and paralysed by a sudden spell of cold weather; this greater independence of the environment, which is characteristic of elevation of type, has been achieved by the warm-blooded animals, including man. Such animals are often spoken of as *homoiothermic, i.e.* animals possessing a uniform temperature, in contradistinction to the cold-blooded animals, which are *poikilothermic* and possess a temperature varying with that of their surroundings.

Amongst the warm-blooded animals, the body temperature may be slightly different according to the species. In birds, it is generally from 39° to 40° C.; in mammals it varies from 35° to 40° C., *e.g.* on the average in health—horse, 37·8°; cow, dog, cat, 38·6°; pig, 39·2°; sheep, 40·0° C.

THE BODY TEMPERATURE IN MAN

In any animal an active tissue, *e.g.* a contracting muscle, must be warmer than an inactive tissue, and this again than a tissue from which heat is being rapidly abstracted, such as the skin. Owing, however, to the high specific heat and thermal conductivity of water, and to the rapidity of the circulation of the blood, the temperatures of the internal organs tend to become equal. The temperature of man is usually taken in the mouth, rectum, or axilla. The temperature, of course, varies with the position at which it is taken, being in man, about 0·5° C. higher in the rectum than in the mouth. In the mouth the temperature is liable to fluctuation with the rate of breathing and with loss of heat through the cheeks. In order to determine the temperature in this situation, the mouth should first be kept shut for a few minutes, then the bulb of the thermometer inserted under the tongue and the lips kept closed on the stem of the thermometer for one to five minutes. The temperature of the skin varies considerably according to position, vascularity, clothing, &c. With unclothed subjects the head and neck are warmest (average 35° C. in a room at about 24° C.), and the foot the coolest (29° C.).* Except in cases where the cutaneous vessels are much dilated, the temperature of a thermometer in the axilla takes a considerable time to rise to that of a thermometer in the mouth; it should never be left less than ten minutes in this situation. The following Table represents the temperature of superficial parts of the body:

SURFACE	
Skin covered with clothes	33° to 35° C.
Naked skin in bath at 5° C.	17° C.
„ „ „ 25° C.	26·5° C.
MUSCLES 12 MM. BELOW THE SURFACE	
In bath at 5° C.	36·3° C.
„ „ 25° C.	36·9° C.

The prevalent statement that the normal temperature is 98·4° F. (36.9° C.) is meaningless; most young adults when at rest have temperatures lower than this, usually about 97·5° F. (36·4° C.) on the average during the day †; the fluctuations are definite, usually about ± 1° F. The temperature is higher in females and in young individuals than in males and older ones. During the menstrual periods there is a fall of temperature.

* FOGED. *Skand. Arch. f. Physiol.*, 1932, 64, 251.

† PEMBREY in "Schäfer's Text-book of Physiology," Vol. I., p. 785, 1898. LYON and WALLACE. *B. M. J.*, May 28th, 1932, p. 980; PATON. *B. M. J.*, July 23rd, 1932, p. 142.

The body temperature of man shows variations over about a degree, according to the time of day at which the temperature is taken. The highest temperature is obtained about six or seven in the evening, and the lowest at about four or five o'clock in the morning. With these diurnal changes in temperature are associated parallel oscillations in the rate of metabolism, as shown by the utilisation of oxygen and the elimination of carbon dioxide. They are probably determined by the changes in the movement and tension of the muscles occurring during the waking hours. If the habits of a man or animal be reversed, so that he sleeps in the daytime and performs his normal vocation by night, it is possible partially to reverse also the direction of the diurnal variations in temperature.* The temperature may also be affected temporarily by various acts, such as the taking of food, hot or cold baths,† or muscular work‡; the influence of the latter factor is often considerable. Pembrey found that marching led to a considerable rise in temperature, which is apparently responsible for the discomfort observed under such circumstances. Such a change in the body temperature is merely temporary in its effects.

Just as there are limits to the power of the organism to regulate its temperature when there is an excessive formation of heat within the body, so there are limits to the capacity to regulate the temperature when severe alterations occur in the temperature of the external medium. Thus if the body is subjected to excessive external cold, or if the loss of heat be increased by absence of clothes, by depriving an animal of its fur or by immersion in a cold bath, the temperature of the body may sink continuously. In the higher animals this fall of temperature is very soon followed by paralysis of the highest nerve centres and by loss of consciousness; the respiration is slowed and the blood pressure falls. If the temperature does not fall too low, it is possible to revive the animal or man by checking the loss of heat, and by supplying artificial warmth. Recovery has, in fact, been observed in men in whom, as a result of exposure, the body temperature had fallen to 24° C. In the same way, exposure to extreme heat, especially if associated with muscular exercise and increased production of heat in the body, may cause a rise of the body temperature. A man or animal whose temperature is raised above the normal is said to be in a state of *pyrexia*. A rise of 2° or 3° C. is associated with all the phenomena which characterise fever, *i.e.* quickening of pulse and respiration, malaise, headache, and loss of muscular power. If the temperature rise to a greater degree than this, the patient may lose consciousness, and death ensues at a temperature of about 44° C.

Regulation of Body Temperature. The very small variation in the body temperature of mammals connotes an equalisation of heat production and heat loss by adjustment of one or both. It will be convenient to deal under separate headings with these two methods of regulation. But first a prevalent misconception must be cleared up. This is the belief that a raised temperature means increased heat production or diminished heat loss, or both.

A *rising* temperature means that the rate of heat production is greater than that of heat loss, and conversely for a falling temperature. But a steady *raised* temperature means that once more heat production and loss are balanced, though the regulator is, for some reason, set to a different level; the raised temperature by itself tells us nothing about the rate of production and loss except that they are equal.

* BENEDICT. *Amer. J. Physiol.*, 1904, **11**, 145.
† HILL and FLACK. *J. Physiol.*, 1909, **38**, Proc. lvii.
‡ MACKEITH *et al.* *Proc. Roy. Soc.*, 1923, B. **95**, 413.

THE REGULATION OF HEAT PRODUCTION

The reactions mainly responsible for heat production in the body are those associated with oxidation. In these processes all the organs participate, the most important, especially in relation to the regulation of heat production, being the skeletal muscles. These represent more than half the total weight of the soft tissues of the body, and even during rest they are the seat of oxidative processes and therefore of heat formation. Heat formation varies with the state of tone of the muscles and is largely increased with every active contraction, since at least 75 per cent., and usually more, of the energy used up by the muscle is set free as heat.

It is probable that, weight for weight, the glands are still more effective as heat producers. The liver, and the blood flowing from the liver, have been stated to present a higher temperature than any other part of the body.

If the oxygen utilisation of the body is increased, the heat production is also raised, and if the heat loss is not also proportionally increased the body temperature will be increased until loss does equal production. Thus the intravenous injection of 0·02 g./kg. of dinitro-α-naphthol may cause the body temperature of dogs to rise to 45° C. and of pigeons to 50° C. ; the oxygen consumption in such cases may be increased several-fold.*

The skeletal muscles, if separated from their centres in the cord, become flaccid and rapidly atrophy. The heat production in the muscles is, therefore, also dependent on their connection with the central nervous system. If this connection be severed, either by curare or by section of the cord, or if the reflex play of impulses on the muscles be abolished by anæsthetics, the animal will react like a cold-blooded animal.† The total metabolism of the body and the total production of heat then sink to a minimum, and are diminished by application of cold, or increased by application of warmth, to the surface of the body. On the other hand, in the intact mammal, moderate changes of temperature in the environment reflexly provoke changes in the *opposite* direction. This effect is called the *chemical regulation* of temperature. Thus, exposure to cold increases, and to heat diminishes, the metabolism and heat production of the body. Though these changes no doubt affect the state of tone of the muscles, and cold may also produce shivering, moderate exposures to cold lead to similar increases of metabolism by other means. Probably a discharge of adrenaline from the suprarenals is one of the chemical factors which brings about an increase in general metabolism under these circumstances.‡ It has been found that the basal metabolism is considerably raised by adrenaline injection §; after removal of both suprarenals the body temperature is lowered and regulation is less precise. Further, certain drugs, *e.g.* tetrahydro-β-naphthylamine, which cause great rise of body temperature, cause general sympathetic excitation and discharge of adrenaline from the suprarenals ‖; again, exposure to cold causes reduction of the adrenaline content of the suprarenals.¶ Nitro-bodies, however, cause a rise of

* HEYMANS and BOUCKAERT. *Arch. inst. pharm.*, 1928, **35**, 63 ; EULER, *ibid* , 1932, **43**, 67.

† PFLÜGER. *Pflüger's Arch.*, 1878, **18**, 305 ; KENNAWAY and PEMBREY. *J. Physiol.*, 1912, **45**, 82.

‡ HARTMANN and HARTMANN. *Amer. J. Physiol.*, 1923, **65**, 612 ; CANNON *et al. Amer. J. Physiol.*, 1927, **79**, 466.

§ BOOTHBY and SANDIFORD. *Amer. J. Physiol.*, 1919, **51**, 200 ; AUB *et al. Amer. J Physiol.*, 1922, **61**, 349.

‖ MUTCH and PEMBREY *J. Physiol.*, 1911, **43**, 109 ; ELLIOTT. *J. Physiol.*, 1912, **44**, 379; CRAMER. *Brit. J. Exp. Path.*, 1920, **1**, 31.

¶ CROWDEN. *J. Physiol.*, 1929, **68**, 313

temperature after removal of the suprarenals, and must act directly on the tissues.

An increased activity of the thyroid has also been claimed to result from exposure to cold; this would also raise the metabolic rate.

The effects of variations in the external temperature on the metabolism of warm-blooded animals are well shown in the experiments from which the Tables given below are taken, on the CO_2 output in the ornithorhynchus (Martin) and the oxygen usage of a rabbit and of a curarised dog.

ORNITHORHYNCHUS. WEIGHT, 693 GRAMMES; SURFACE, 876 SQ. CM.

Temperature of environment	Temperature of animal	Difference in temperature, animal and environment	CO_2 per hour, in grammes	CO_2 per hour per 1000 sq centims, in grammes
5	31·8	26·8	1·090	1·244
10	32·0	22·0	0·722	0·825
20	32·6	12·6	0·405	0·463
32	33·6	1·6	0·336	0 383
35	35·3	0·3	0·377	0·430

Rabbit (Pfluger)		Curarised Dog (Krogh)	
Rectal temperature °C.	c c Oxygen per Kg and hour	Rectal temperature °C.	c c Oxygen per Kg and hour
22	457	14·1	134
26	608	22·7	300
37·5	888	28	495
37·6	839	32·2	620
38	763	37·2	812
38·8	738	39·9	700

In the first animal, where the regulation of the temperature of the body is effected almost entirely by changes in heat production, the effect of warming the environment of the animal on the CO_2 output is conspicuous. It will be noticed that the CO_2 per hour sinks continuously with rising temperature, up to 32° C. When the temperature of the chamber was raised to 35°, the temperature of the animal rose considerably, *i.e.* the regulatory mechanism was failing, so that the same effect was produced on metabolism as is observed in working with cold-blooded animals This latter change, though less marked, is observed on exposing the rabbit to a gradually rising temperature. Here, however, the process of regulation is aided by alterations in the heat loss as well as in the heat production (Fig. 614). If the animals be observed whilst subjected to changes of temperature, it will be evident to anyone that the regulation is associated with changes in muscular activity At 30° to 35° C. the animals will lie perfectly flaccid, breathing rapidly, or they may go to sleep. On cooling, they at once become more vigorous and perform active movements in their cage. The same effects of changes in the external temperature, familiar in ourselves, are indications of the important part played by the muscles in determining the heat production of the body. As a rule, the immersion of a man in a cold bath for a minute or two considerably increases his oxygen usage. It is possible, however, to sit in a bath, and, by an act of the will, keep all the muscles in a

state of relaxation. Under these circumstances the temperature of the body rapidly falls, and with it the rate of metabolism, as judged by the oxygen consumption of the subject.

This process of adjustment of the body temperature by variations in the heat production is extravagant of energy if the difference in temperature between animal and environment is considerable. In the very perfect adjustment of the temperature which is present in the higher mammals, regulation of the heat loss plays a greater part than regulation of heat production. The economy of adjustment by heat loss is well shown if we compare in echidna and rabbit respectively the percentage alteration in CO_2 production, when the difference in temperature between animal and environment alters

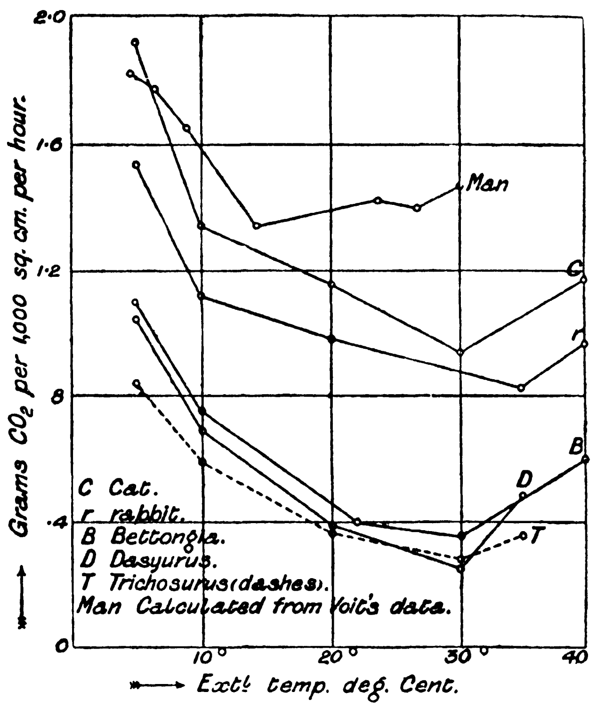

FIG. 614. Effect of Variations in the External Temperature on the CO_2 Output (per 1000 cm.[2] Body Surface) of Warm-blooded Animals. (C. J. MARTIN.)

from 10° C. to 20° C. This is, for echidna 72 per cent., for other mammals 16 per cent. In echidna, variations in heat loss can be practically neglected, so that the whole work of regulating the body temperature falls on the heat production. As soon as the external temperature falls below a certain degree, the mechanism fails, the animal's temperature falls, and it passes into a state of hibernation.

THE REGULATION OF HEAT LOSS

In all climates, except under certain exceptional conditions, the temperature of the warm-blooded animal is higher than that of his environment, so that there must be a constant loss of heat from the surface of the body. In mammals in the arctic regions, and in those which have adopted an aquatic existence, the thick layer of fat which underlies the skin protects the active portions of the body from excessive loss of heat. In most terrestrial animals,

the loss of heat is also diminished by fur and feathers, and in ourselves the same office is performed by clothes. The value of these different coverings depends on the fact that air is a very bad conductor of heat. With a naked man, the layer of air immediately in contact with the surface of the body is warmed and rises, its place being taken by fresh cold air (convection). Loss of heat is increased by a draught. In clothes, it is the air enclosed in the meshes of the garments and between their different layers that plays the greater part in preventing loss of heat.

In addition to the loss of heat by *convection*, heat is also lost by *radiation* to cooler objects. This loss is also reduced by clothing. Since the material of which clothes are made does not allow the passage of radiant heat, they absorb the heat leaving the body. It is a familiar experience that a multiplication of garments is more effective in retaining the heat of the body than merely increasing the thickness of the individual garments.

Since the loss of heat depends on the difference of temperature between the surface of the body and the surrounding air or objects, it will be largely affected by the surface temperature, and therefore by the amount of blood flowing through the skin. The blood flow through the skin is under the control of the central nervous system, through the vaso-constrictor and vaso-dilator nerves, and when the skin is cooled it is by altering the size of the cutaneous vessels that the central nervous system chiefly acts in regulating heat loss.* In cold weather, or when the heat production in the body is low, the vessels are constricted, the skin is cold, and the heat loss is small. In the extremities of the body, although at first there is some vasoconstriction, excessive skin cooling, with ensuing tissue damage, is probably prevented by the opening up of the arterio-venous anastomoses. In this way the skin surface is flushed with warm blood so that its cooling is delayed. Hence, after handling snow or ice the skin becomes red, warm and hyperalgesic.† The anastomoses are controlled, probably centrally, by constriction or dilatation of the arterioles.‡ The reaction is closely related to the hyperalgesia in which the "nocifensor nerves" play a part, and also to the spreading flare seen on local damage to the skin.

On the other hand, if the temperature of the surrounding air is high, or a large amount of heat is being produced in consequence of muscular exercise, the vessels are dilated and the skin is hot. In hot weather there is a redistribution from the muscles, where the blood is not required, to the skin, where a considerable circulation is necessary.

The body temperature can be maintained normal even in an atmosphere with a temperature much higher than 37·0° C., and this in spite of the fact that the production of heat in the body is still going on. This is because there is a profuse secretion of sweat on the surface of the body. In the evaporation of the sweat, especially if aided by a draught of air, a large amount of heat becomes latent and is abstracted from the body, which is therefore kept at a temperature below that of the surrounding atmosphere. The evaporation of 1 gramme of water at body temperature absorbs 0·58 Cals., so that evaporation of 120 grammes of sweat would lower the body temperature of a 70 kg. man about 1° C. If, as happens in rare cases, a man has no sweat glands,§ or if evaporation be impeded by placing him in a hot atmosphere already saturated with aqueous vapour, the temperature of the body runs up rapidly and death may ensue from hyperpyrexia or heat-stroke.

* PICKERING. *Heart*, 1932, **16**, 115.
† LEWIS. *Heart*, 1930, **15**, 177, 351.
‡ GRANT and BLAND. *Heart*, 1931, **15**, 385; GRANT, BLAND and CAMP. *Heart*, 1932, **16**, 69.
§ RICHARDSON. *J. Biol. Chem.*, 1926, **67**, 397.

Possibly the reports that death may result from entirely coating the skin with an impervious layer may indicate a similar condition. Cases are on record of boys being varnished and gilded in order to feature in carnivals and processions, *e.g.* one in Rome in 1473 and one in Florence in 1513, with fatal results. With a wet-bulb temperature even up to 79° F. moderate work can be carried out if the air is kept in motion; with still air work is not possible at that wet-bulb temperature; a wet-bulb temperature of 85° F. is insupportable even when at rest and with movement of the air. Although a man can stand exposure to a temperature of 200° or even 250° F. for an appreciable time, provided that the air is dry, a temperature of 89° F. is rapidly fatal if the air be saturated with moisture. The same mechanism comes into play when the heat production in the body is very largely increased, as by violent exercise. Under these conditions, a man may sweat profusely when the temperature of the surrounding atmosphere is below 0° C.

The main regulation of heat loss thus takes place by the control, through the nervous system, over the cutaneous circulation and the sweat glands. Besides these channels of heat loss, others may play an important part under certain conditions. Heat is lost to the body in warming the food and air which are taken in. It is also lost in respiration in the evaporation of water and the setting free of CO_2 from watery solution into the expired air. The following estimate represents the proportion of losses daily in an adult man by these different ways:

A. Warming the Food and Air

		Cal.
(1) 1500 g. water drunk at 15° C. and warmed to 37·5°—raised therefore 22·5°	=	33·75
(2) 1500 g. food eaten at 25° C. (mean) and warmed to 37·5°—raised therefore 12·5; specific heat 0·8	=	15 00
(3) 15,000 g. (= 11,500 l.) air respired at 15° C. and warmed to 37·5°—raised therefore 22·5°; specific heat 0·237	=	79·95
		128·70

B. Loss of Water and CO_2 in the Breath

		Cal.
(4) It is assumed that the inspired air is half saturated with water vapour at 15° C. and that the expired air is fully saturated at 37·5° C. Approximately 450 g. of water would be given off therefore in the form of vapour from the respiratory passages; the latent heat of the water vapour is 0·58 Cal.	=	261·00
(5) The absorption of heat in the liberation of CO_2 from the lungs (800 g.); 0·134 Cal. per g.	=	107·20
		368·20
From above		128·70
Total		496·90

Estimating the total day's loss of an adult man at 2400 Cal., the above sum represents only about 20 per cent. of the total. The remaining 80 per cent. (in round numbers) takes place through the skin.

If we estimate the total heat loss of an adult man at 2400 Calories, we may say that about 5 per cent. of the total heat loss takes place by warming the food and air, about 15 per cent. by the evaporation of water and CO_2 in respiration, and about 80 per cent. by radiation and convection and the evaporation of sweat from the skin. The proportion represented by the last factor will increase very largely in the presence of a high external temperature, or of an excessive heat production in consequence of violent muscular exercise.

The chief physiological responses to exposure of the body to heat are :—increase of respiratory ventilation, with rise of blood *p*H, excretion of alkaline urine, increased pulse and circulation rate, sweating, dilatation of arterioles and capillaries in the skin, usually a fall of arterial pressure, an increase of blood volume, rise of body temperature, and in extreme cases rise of basal metabolic rate. After prolonged exposure, say for several days, acclimatisation occurs. Sweating then occurs more easily, and the sweat is more dilute. The urine becomes more concentrated and may at times be free from chlorides.

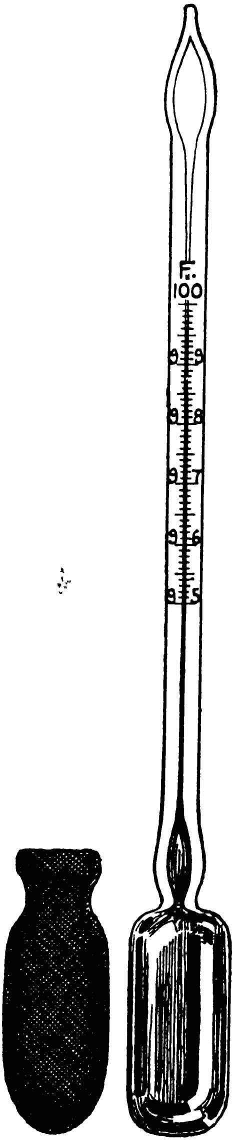

FIG. 615. The Kata-thermometer. On the left the cotton glove for use as a wet indicator. (After L. HILL.)

Insensible Perspiration. It has been known since the work of Sanctorius in 1614 * that a person slowly loses weight between meals. This loss is of the order of 30 g. per hour in subjects in a basal metabolic state. It is chiefly due to loss of water by skin and lungs, and only to the extent of about 10 per cent. to the difference between oxygen intake and CO_2 output. It varies according to the metabolic rate, as would be expected, since it represents the means by which about one-quarter of the body heat is lost. About one-quarter of the total loss from the skin takes place from the hands and feet, which lose moisture at about three times the rate found for equal skin areas elsewhere.†

Influence of Atmospheric Conditions.—The cooling power of the air upon the skin varies over an extremely wide range, since it depends upon differences of temperature, humidity, and rate of movement of the air. There is further the influence of the absorption of radiant heat from without, by skin or clothing.

The conditions indoors and outdoors, are, under ordinary circumstances, fundamentally different, the cooling and evaporative powers being greatly reduced indoors, owing to the stillness of the air.

The ordinary thermometer gives us no indication of the physiological aspects of the atmospheric conditions. The wet- and dry-bulb thermometer gives a little more information, because the evaporative power of the atmosphere is indicated by the depression of the wet-bulb instrument. A still better and very practical instrument is the Kata-thermometer (see Fig. 615) introduced by Leonard Hill.‡ This measures the cooling power of the air on surfaces, wet or dry, at body temperature. It is an alcohol thermometer with a large bulb and a stem graduated at 95° and 100° F. It is warmed up to about 105°, and then the time taken to cool from 100° F.

* SANCTORIUS. 1614, *Medicine Statica.*

† BENEDICT and WARDLAW. *Arch. Inst. Med.*, 1932, **49**, 1019.

‡ HILL, GRIFFITH and FLACK. *Trans. Roy. Soc.*, 1916, B. **207**, 183 ; HARGOOD-ASH and HILL. *M. R. C. Rep.*, 1923, **73**.

to 95° F. is noted. Each instrument is previously calibrated * and a numerical factor supplied with it. This factor, divided by the time in seconds taken to cool over the specified range, gives the rate of cooling in milli-calories per square centimetre of surface per second. This varies according to the temperature, humidity and velocity of movement of the air. The dry Kata-thermometer gives us the heat lost by radiation, conduction and convection. The wet Kata-thermometer, in which the bulb is covered with a wetted cotton glove, gives us, in addition, the heat lost by evaporation. With this instrument, valuable investigations on the physiological aspects of climatic conditions, and of the influence of clothing on the cooling rates of surfaces, comparable with that of the body, have been made.

Effective Temperature. Another method of assessing climatic conditions † is by the estimation of what is called the effective temperature. This has been empirically devised by direct observation on human subjects and takes into account temperature, humidity and convection in the air.

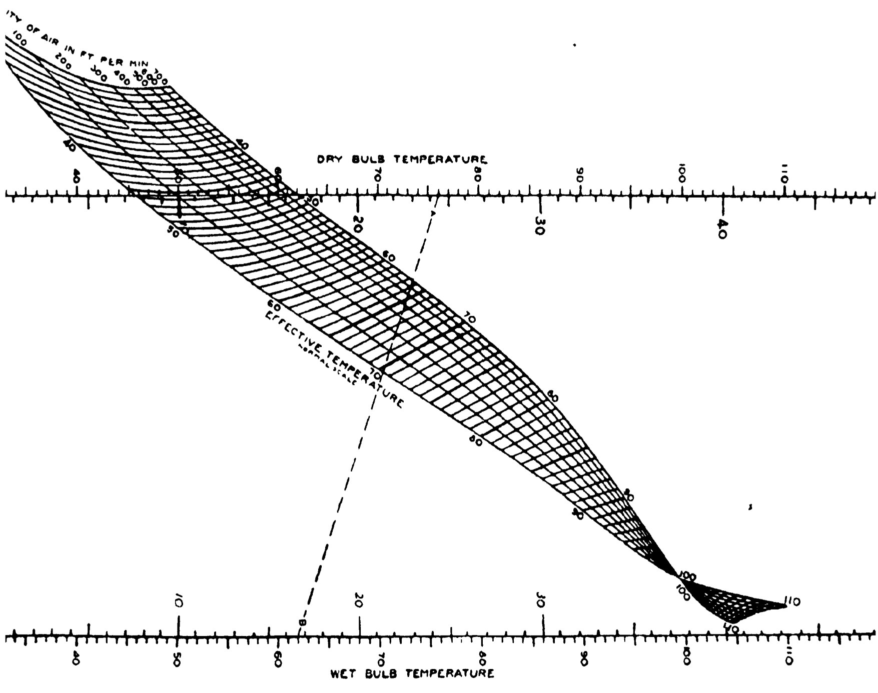

FIG. 616. Nomogram for determination of effective temperature on men normally clad and doing light work. (HOUGHTON, TEAGUE and MILLER. *J. Amer. Soc. Heat. Vent. Engrs.*, 1926, **32**, 315, 473.)

The effective temperature of an atmosphere is the temperature of a still, saturated atmosphere which would produce the same feeling of temperature as the atmosphere in question. It is found by determining dry bulb and wet bulb temperatures and average air velocity. Then by reference to a nomogram (Fig. 616) the effective temperature is read off directly. Thus a dry-

* ANGUS. *J. Industr. Hyg.*, 1924, **6**, 20.
† LEE and COURTICE. *Trans. R. S. Trop. Med. Hyg.*, 1940, **33**, 601.

bulb temperature of 76° F. (24·5° C.) and a wet bulb of 62° F. (16·5° C.), with air velocity of 100 ft. per minute, gives, as shown by dotted line AB, an effective temperature of 69° F.

THE NERVOUS MECHANISM FOR HEAT REGULATION

The accurate balance between heat production and heat loss, which is responsible for the nearly constant temperature of mammals, involves co-ordination by the higher levels of the central nervous system. A "decerebrate" animal (*i.e.* one with the brain anterior to the anterior colliculi removed) cannot regulate its body temperature, but an animal merely deprived of its cerebral cortex can do so; so also can one deprived of all brain substance anterior to the middle of the optic thalamus.* Reflex shivering as a response to cold is also controlled, not from the spinal cord, but from somewhere anterior to the mid-brain.†

There is good evidence that the important function of integrating the various mechanisms concerned with temperature regulation is located in the hypothalamus.‡ Both heat production and heat loss are there controlled. The lower centres for heat loss must be placed in the medulla and cord, at any rate so far as concerns control of heat loss by alterations in the blood supply to the skin, or by the secretion of sweat. The same is true for the heat production arrangements, such as shivering, vaso-constriction, metabolic changes, etc. But these lower centres can only function in a co-ordinated manner when under the higher control of the hypothalamus.

If the anterior part of the hypothalamus is warmed, the processes causing heat loss, *e.g.* vasodilatation, panting, sweating, etc., are put into action §; this suggests that some nuclei here act as thermoregulators, responding directly to a change in temperature of the blood. Electrical stimulation of the hypothalamus may also produce similar responses, while lesions of the anterior hypothalamus lead to a loss of power to withstand high temperatures.‖ It may be concluded therefore that the general response to rise of temperature is directed from the anterior hypothalamus.

Lesions of the posterior part of the hypothalamus tend to cause subnormal body temperatures ¶; animals so operated exhibit vasodilatation, and fail to shiver as the temperature drops. It is inferred, therefore, that responses to falling temperature are controlled mainly by the posterior or sympathetic group of hypothalamic nuclei. The response of shivering is certainly co-ordinated in the hypothalamus, and the efferent paths covered are not the pyramidal tracts, but probably tectospinal or rubrospinal paths.**

In many warm-blooded animals, the ability to maintain a constant temperature is not fully developed until some time after birth. Pembrey †† has shown that, in the guinea-pig and chick, in which the nervous system is fully functional at birth, the heat-regulating mechanism is also completely adequate, whereas animals such as rats, pigeons, or the human child, which are born in a helpless condition, only acquire the power of regulating their own temperature some time after birth. As we should expect, the development of the power of regulating heat production runs *pari passu* with the acquisition of control by the nervous system over the muscles of the body.

* ISENSCHMID and KREHL. *Arch. f. Exp. Path. u. Pharm.*, 1912, **70**, 109.
† SHERRINGTON. *J. Physiol.*, 1924, **58**, 405.
‡ BAZETT *et al.* *Arch. Neurol. & Psych.*, 1933, **30**, 728.
§ MAGOUN *et al.* *J. Neurophysiol.*, 1938, **1**, 101.
‖ RANSON, FISHER & INGRAM. *Arch. Neurol. Psych.*, 1937, **38**, 445.
¶ CLARK, MAGOUN and RANSON. *J. Neurophysiol.*, 1939, **2**, 61.
** UPRUS, GAYLOR and CARMICHAEL. *Brain*, 1935, **58**, 220.
†† PEMBREY. *J. Physiol.*, 1894, **17**, Proc. xxvii.

Under certain conditions the heat regulation is interfered with, and a condition of *fever* results, in which the body temperature remains for hours or days at a high level. The condition is produced under the influence of certain infections, or by the injection of bacterial toxins, &c. In most, if not all, of these febrile conditions the raised temperature is due, not to increased production, but to diminished loss of heat, and it would seem that the heat regulating " centre " has become " set " at too high a level. There is a retention of water in the tissues, concentration of the blood, absence of sweating and reduction of urinary and other secretions, so long as the fever persists; according to some this water retention is a primary phenomenon and causes the rise of temperature.* Hence it is possible to produce hyperthermia by causing blood concentration by the injection of strong solutions of sugar into the blood stream. The heat production is not much raised in fevers, it is rather a lowering of heat loss that is the responsible factor.

Antipyretic drugs probably act by paralysing hypothalamic centres, as also do barbiturates.

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* BALCAR, SANSUM and WOODYATT. *Arch. Inst. Med.*, 1919, **24**, 116.

BOOK VIII

THE ENDOCRINE ORGANS AND REPRODUCTION

A. The Endocrine Organs

B. Reproduction

A. *THE ENDOCRINE ORGANS*

CHAPTER L

THE ENDOCRINE ORGANS

WHEN, in a primitive organism, the reaction of distant parts of the body to a change occurring in any one part depends on the diffusion of some substance from the stimulated part, the total reaction must require a considerable time for its full development. A much more effective method of correlation was acquired by the evolution of a nervous system, but this second mode of correlation did not do away with the necessity for the more primitive method. Even in the higher animals, when rapidity of reaction is not required, we find adaptations carried out in response to some change in distant parts of the body, the message being chemical and not nervous in character (*e.g.* the secretin mechanism for pancreatic secretion).

When we speak of the chemical correlation of the activities of the different parts of the body, it is important not to confuse processes which have little or nothing in common. In one sense, we may say that every cell in the body is chemically connected with, and dependent on, all the other cells in the body. Thus, one organ may receive and modify any substance or foodstuff so as to prepare it for more ready assimilation by other tissues. It may shield other tissues from the poisonous effects of certain waste products, either by converting them into harmless substances or by excreting them from the body.

We are probably justified in treating apart a group of phenomena in which the substance transmitted from one part of the organism to another is significant almost entirely as an excitatory agent, and has little or no value as a source of energy. When the adaptation to a change at A consists in increase or diminution in the activity of an organ B, the change in B can be evoked by the production at A, as a direct consequence of a stimulus, of a specific chemical substance, which passes in the circulating blood to B, where in its turn it will produce the required state of action. Such chemical messengers may be called, in general, *autacoids :* when the effect is to cause excitation they may be called *hormones*, from ὁρμάω, 'I excite.'* We have already met with several examples of such bodies. The same autacoid may exert a stimulating action on one organ and a depressant one on another, however. Usually they are called hormones, irrespective of the direction of their action. It may be interesting here to consider what must be the character of substances which fulfil the part of chemical messengers.

(1) Since they must be carried by the blood stream to the reacting organ, they must, in most cases, be susceptible of easy passage through the walls of the blood vessels, if they are to excite a reaction within a fairly short space of time. This consideration would necessitate their molecular weights being comparatively low, and, actually, several of them have a comparatively simple chemical and molecular constitution. Some, however, are complex, *e.g.* insulin is a protein.

(2) Hormones fall into two rough groups according to whether their actions are fugitive or lasting. In those of the former group, the chemical

* STARLING. Croonian Lect. R.C.P., 1905; JAYLE. *Bull de l'académ. de Méd.*, 1937, **117**, 73.

messenger must excite a state of activity (or of inhibition) in response to a change in some other part of the body, *e.g.* when adrenaline is thrown out from the suprarenals in states of sympathetic excitation. When the primary change passes away, the action of the hormone should also disappear. On this account it is necessary that the hormone should either be susceptible of easy destruction, by oxidation or otherwise, in the fluids of the body, or be readily excreted, so that its action may not be continued or even augmented, indefinitely.

In the other group of hormones, which are more stable, the effect is protracted, appearing and disappearing slowly, as, *e.g.*, is the case with the hormone of the thyroid, with the sex hormones, and with some of the pituitary hormones.

(3) They must not be *antigens*, *i.e.* their injection into the blood stream must not involve the production of an antibody. If this were the case, the hormone, on entering the blood stream, would meet its antibody and would be unable to exert any effect on the appropriate reacting organ.

The production under experimental conditions of anti-hormones to certain of the active principles of the pituitary, when these are injected, is an apparent exception. The hormones in question are proteins, and some investigators claim that the production of the anti-hormones is only observed, or is most evident, when the pituitary material is derived from a different species of animal from that receiving the injection. Whether these anti-hormones have any physiological significance is therefore at present an open question.

In previous chapters, we have already come across several examples of correlated activities of different tissues, effected by chemical means. Thus *secretin*, which is heat stable and diffusible but is easily destroyed by oxidation, is absorbed into the blood and carried to the pancreas, where it acts as a specific stimulus for the secretory cells. This was the first discovered example of a chemical reflex, *i.e.* an adaptation effected by chemical means rather than by impulses passing along the nerve channels.

There is one class of organs in which the production of hormones is the only function with which we are acquainted. These are included under the term *endocrine organs*. As examples. we may cite the suprarenal bodies, the thyroid and parathyroids and the pituitary body. Their functions, however, are rarely, if ever, single. Thus we have the cortex and medulla of the suprarenal each with distinct endocrine functions; similarly the anterior and posterior parts of the pituitary body, while the thyroids and parathyroids are often as close in situation as they are remote from one another in function. In other cases, an organ may have a twofold function. Thus, the pancreas gives an external secretion, the pancreatic juice, and an internal secretion, insulin, which, passing into the blood, exercises an important influence on the metabolism of carbohydrate. Other instances of these chemical correlations may be cited, *e.g.* the secretion of gastric juice, by the production, in the pyloric mucous membrane, of a gastric hormone. In the reproductive system, we have many instances of such chemical correlations.

Methods of Investigation. The investigation of the functions of the endocrine organs consists essentially in studying the effects, on the various functions of the body, of augmenting and of diminishing the amount of active principle of the organ in question. There are several directions along which this information may be obtained. Thus, in certain diseases, the endocrine organ in question produces less of the active principle than it does in the normal state, or, conversely, in conditions associated with overgrowth or overaction of the gland, it may produce more. Careful study of the

clinical effects of the hypo- or hyper-function of the particular endocrine organ therefore throws light on the functions which it normally performs.

The effects of lack of the endocrine principle can be studied, in experiments on animals, by the removal by operation of the gland in question, such operations being sometimes performed in a series of stages, so that the resultant change is a gradual one. Then, having produced the effects of lack of the active substance, we may attempt to make good this lack by the administration of the substance, either by feeding with the gland, by administering extracts of it intravenously or by subcutaneous injection (insoluble substances being given suspended in oil); or by the surgical grafting of a portion of the gland from another animal of the same species. We can also, of course, study the effects of similar administration of the active principle to the normal animal or man, and thus learn the physiological effects of an excess of the hormone.

Next, chemical investigation seeks to isolate and to ascertain the nature of these active principles, with the view to their ultimate synthesis in the laboratory, an end which has already been achieved in several instances. It is also essential to find some method by which the amount of the active principle present in a tissue or extract may be estimated. This is usually a method of biological assay, as, *e.g.*, in the case of the standardisation of insulin preparations, as chemical methods are not delicate enough. A unit of biological activity is decided upon (often by international agreement), and, when possible, this is related to a known weight of the pure active substance.

The presence, in extracts of tissues, of substances which exert definite physiological effects when administered in appropriate ways, is, however, no proof that the organ in question normally produces that substance for the benefit of the whole body, or that the substance is ever passed, as such, from the organ into the blood or lymph. Strict proof would require evidence that the organ in question, (*a*) contained an active chemical substance, which (*b*) is essential to some other parts of the body, so that (*c*) definite effects would ensue, if the tissue which forms this active principle were all removed, but (*d*) these effects would disappear if the active principle was suitably administered; (*e*) the active substance should be demonstrable in the blood or in the lymph leaving the organ in which it is produced.

THE SUPRARENAL BODIES

The suprarenal bodies, or adrenals, in mammals are two small masses lying on or near the upper or oral pole of the kidneys. They consist of two parts, the cortex and the medulla. The cortex is composed of cells arranged in columns or in a reticular fashion. According to the arrangement of its cells the cortex is divided into three zones, the zona glomerulosa, zona fasciculata, and zona reticularis. The cells themselves are distinguished by the large amount of granules they contain, which probably consist of lecithin and cholesterol compounds. These granules stain with osmic acid, and are dissolved out by turpentine. The cortex cells stain black with silver nitrate on account of their high content of vitamin C. In some animals, *e.g.* the guinea-pig, the cells, especially towards the inner part of the cortex, contain many yellow pigment granules.*

The medulla, much less extensive than the cortex, presents irregularly shaped cells, the outlines of which are but slightly marked. These cells contain granules which stain darkly with chromates and give a green colour with salts of iron. It is hence easy to delimit the area of the medulla in any section of a gland which has been hardened in a fluid containing chromates. The substance giving this reaction is known as

* CRAMER. *J. Physiol.*, 1918, **52**, Proc. viii.

chromaffine substance.* Fine osmic-staining granules are also present in abundance in these cells, but are *not* dissolved out by turpentine. All these reactions are due to the presence of adrenaline, The suprarenals are richly supplied with blood, especially in the medullary part, the cells of which impinge directly on the endothelial lining of dilated capillaries. The blood supply is probably the most abundant in the whole body. They also receive an abundant nerve supply from the sympathetic system (splanchnics), the nerves forming a thick meshwork, especially in the medulla; this contains also a few ganglion cells, which may either be isolated, or combined to form small ganglia.

A study of the development of the suprarenal glands shows that we have here to do with two distinct tissues, which have different origins and different functions in the body Whereas the cortex is derived from that portion of the mesoblast, the 'intermediate cell mass,' from which the mesonephros is also developed, the medulla is produced by an outgrowth from the sympathetic system, and may indeed be said to consist of profoundly modified nerve cells. In many fishes, these two elements of the suprarenal gland remain separated throughout life, the cortex being represented by a series of paired inter-renal bodies lying on the front of the spinal column, and the medulla by a number of collections of chromaffine cells, lying in close juxtaposition to the spinal nerves.

In some animals, accessory suprarenals are not infrequent in which both cortex and medulla may be represented. In all animals we find masses of tissue, the so-called paraganglia, in close association with the sympathetic system, which present the chromaffine reaction typical of the medulla. Since a watery extract or decoction of these bodies has the same influence, on injection into the blood stream, as an infusion of the medulla of the suprarenal body itself, we are probably justified in regarding these bodies as equivalent to accessory medullary portions of the suprarenal. They have the same origin, the same staining reactions, and the same physiological effect as the latter.

Scattered portions of cortical tissue are also found in many animals, usually near to the gonads.

The functions of the cortex and medulla are quite distinct.

FUNCTIONS OF THE CORTEX

Extirpation of one suprarenal (single epinephrectomy) has no effect beyond that of sometimes leading to hypertrophy, especially of the cortex, of the other one. This may be due to a mutual reaction between suprarenal cortex and anterior pituitary, resulting in increased formation by the latter of the adrenotrophic hormone. The results of extirpation of both suprarenals vary. It is easy to keep old male rats, on suitable diets, alive after total extirpation of these organs, and some rabbits also survive double epinephrectomy indefinitely. To cats, dogs, young rats and guinea-pigs, however, the operation is fatal in, at most, a few days. After recovery from the operation the basal metabolism of the animal, as measured by oxygen consumption, remains normal for a few days and then, when symptoms of weakness appear, falls considerably.† When extirpation is fatal, death occurs suddenly, and is preceded by rapid rise of blood urea and non-protein nitrogen, loss of appetite, imperfect fat and carbohydrate absorption, weakness of muscles, lowering of arterial pressure, and of blood sugar, fall of body temperature, collapse, and a shock-like condition, with great concentration of the blood. It was shown by Loeb ‡ that there is an increased rate of loss of sodium salts by the kidneys after epinephrectomy, the general dehydration and reduction of blood volume is to some extent a consequence of this lowered blood sodium. Since the urea clearance is also reduced, and the excretion of other substances also disturbed, it would appear that there is some interference with kidney function.§ Thus blood

* Vincent. *Proc. Roy. Soc.*, 1910, B. **82**, 502.
† Webster, Pfiffner and Swingle. *Amer. J. Physiol.*, 1932, **99**, 710.
‡ Loeb *et al.* *J. Exp. Med.*, 1933, **57**, 775.
§ Marshall and Davis. *J. Pharmacol.*, 1916, **8**, 525.

potassium, creatinine, phosphate and sulphate are raised, while excretion of injected phenolsulphonephthalein is reduced. Any factor which augments loss of water from the blood aggravates the condition of circulatory collapse, *e.g.* it can be evoked very easily in an epinephrectomised animal by the administration of a small dose of histamine, to which such animals are abnormally sensitive. A similar loss of fluid and electrolytes from the blood occurs in shock.* Conversely, the injection of salt solution into the blood stream, or administration of NaCl by the mouth improves the condition for a while.

Addison in 1855 drew attention to the coincidence of degenerative destruction of the suprarenals with a disease which since that time has been known as Addison's disease. The three cardinal symptoms of this disorder are (1) bronzing of the skin, (2) vomiting, (3) excessive muscular weakness and prostration. The disease is almost invariably fatal. Addison's observations have been amply confirmed since that time, but we are not yet in a position to account for the occurrence of all these symptoms as a result of interference with the suprarenals since it has been impossible experimentally to reproduce the bronzing which is so characteristic of Addison's disease. It is possible that future work may show some connection between the cortex and the destruction of pigment in the body. At present it is only by a process of exclusion that we may guess at a causal relationship between the destruction of the cortex and the bronzing which occurs in Addison's disease.

It is certain that the rapidly fatal effect of extirpation of both suprarenals is to be ascribed to the removal of the cortex and not of the medulla. In the cases where animals survive double extirpation, small masses of accessory cortical substance have been found embedded in the kidney or elsewhere in the neighbourhood of the suprarenals or gonads.

Hypertrophy, or a tumour, of the suprarenal cortex in children is associated with premature sexual maturity † and in women with masculinisation and hirsutism, and also often with a basophile pituitary tumour. Many cases of masculinity (virilism) in women have been dramatically cured by removal of an adrenal tumour; often the male hair is removable in a day or two after operation.

Among many attempts to isolate from the cortex a substance capable of prolonging indefinitely the life of epinephrectomised animals, the first clear success was reached by Swingle and Pfiffner in 1929. They extracted fresh ox suprarenals with alcohol, concentrated the extract and re-extracted it with benzene and various other solvents, and finally obtained a water soluble extract of sufficient purity to permit of administration by intraperitoneal injection.‡ Daily injections of small doses of the extract suffice to maintain in health a previously epinephrectomised dog. Large doses given by mouth are effective.§ When the administrations are withheld the animal rapidly goes downhill, and dies in a few days : but even moribund animals are very soon restored if the dosage is recommenced and sodium chloride given.

Cortical Hormone. From the researches of Kendall,|| of Reichstein, and of Wintersteiner, it has been found that a whole ~~series of active~~ substances, all sterols, are present in the suprarenal cortex. Chief among these are *Corticosterone*, *Desoxycorticosterone*, *Dehydrocorticosterone*, and, in small amounts, *Progesterone* and *Androsterone* (the last two also being known among the

* MacKay *et al.* *Amer. J. Physiol.*, 1935, **112**, 581.

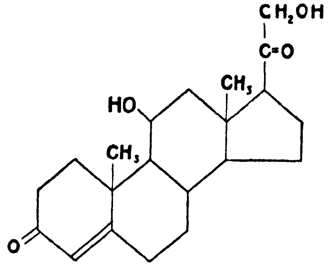
† Bulloch and Sequeira. *Trans. Path. Soc., London*, 1905, **66**, 189.

‡ Swingle and Pfiffner. *Amer. J. Physiol.*, 1931, **96**, 153, 164, 180 ; **98**, 144.

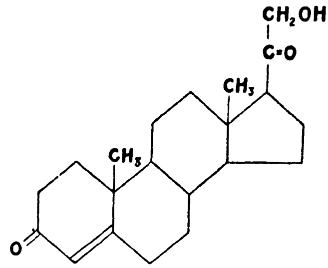
§ Britton, Flippin and Silvette. *Am. J. Physiol.*, 1931, **99**, 44.

|| Kendall *et al.* *J. Biol. Chem.*, 1937, **120**, 719.

sex hormones). They differ in their activity in replacing the hormone lost by adrenalectomy, though all are more or less active.



Corticosterone



Desoxycorticosterone

The essential activity of the cortex is probably to be attributed to the first three hormones named above, which have similar actions. The most active of the cortical steroids is desoxycorticosterone, which is now made synthetically and placed on the market as its acetate. It is more potent than corticosterone in affecting the salt balance, though it has less effect on carbohydrate metabolism.

Clinical trials by implanting the solid hormone under the skin in cases of Addison's disease are still largely experimental, but promising results have certainly been obtained.*

Desoxycorticosterone can completely restore an adrenalectomised animal to normality : so can the other cortical sterols, though in some cases, *e.g.* with androsterone or progesterone, the amounts needed are relatively large

In muscular exercise, and as part of a generalised response of the body to damaging influences, such as trauma, cold, and certain toxic substances, there is evidence of increased activity of the suprarenal cortex, and the administration of cortical hormones has been found useful in combating surgical shock.

The close chemical relationship between the cortical sterols and the sex hormones provides an explanation for the disturbances of sex functions which are found, *e.g.* as virilism in women, in relation to tumours of the cortex. In such cases the inner layers of the cortex were found by Vines to contain granules stainable by ponceau-fuchsin. This material is absent from normal suprarenals, except in fœtal life, up to the end of the first half. The fuchsinophile material appears sooner and persists longer in male than in female fœtuses, and is believed to indicate the presence of androgenic sex hormones. The occurrence of virilism in women seems to be due to the obtrusion of male qualities which, in the normal endocrine balance of the female, are held in abeyance. Substances having actions similar to those of cortical sterols have been recovered from the urine of mares and cows.† Normal women excrete about 8 mg. daily of a substance related to androsterone ; in cortical tumour cases the amount may be increased to 40 mg. or more.

Relations of Cortex with Pituitary Anterior Lobe. After hypophysectomy the inner layers of the cortex, first the zona reticularis and then the zona fasciculata shrink and degenerate, and these changes can be prevented by the injection of suitable extracts of the anterior lobe—the so-called

* Dryerre. *B.M.J.*, 1939 (i.), 971.
† Selye and Dosne. *Lancet*, 1940, **239**, 70.

adrenotrophic fraction. Hypertrophy of the inner cortical layers can be induced by the injection of these extracts into normal animals. The influence of the cortex on the metabolism of carbohydrate, fat and protein are the most affected by the adrenotrophic factor, from which it may be suspected that it is the inner layers of the cortex which secrete the hormones that are concerned with the control of these aspects of metabolism. The outer, and more newly-formed cells of the zona glomerulosa may either secrete enough hormone, or a hormone of the right kind, to exercise control over the salt and water regulation of the body after hypo-physectomy.

Nature of Action. Various explanations have been advanced to attempt to explain why the suprarenal cortex is essential to life, and to find in what the fundamental action of the cortical hormones consists. Apart from any theories, it is evident that it must have some action which affects processes that are quite indispensable to life ; no doubt some of the effects are secondary to this action. Opinions differ as to what the fundamental action is, and depend very much on the clarity with which results emerge. The striking excretion of sodium from the body, together with the accumulation of potassium and loss of water have led to the theory that the fundamental action of the hormones is related to the control of the salt and water balance, and that the other effects, such as those on carbohydrate metabolism are in some way related to this. Another view is that the fundamental effect is on carbohydrate metabolism, and as we have seen in Chapter XLVI, this effect is certainly quite pronounced. Another, and more plausible theory is that the hormones are essential in a still more fundamental way, in that they are concerned with the whole basal metabolic changes of the cells, so that in their absence the whole of the metabolic arrangements are upset. In the process of phosphorylation we have such a fundamental reaction, which lies at the root of many metabolic changes in the body, and Verzár believes that the cortical hormones are indispensable for this. The symptoms of adrenal deficiency, he claims, can be in part cured by the administration of flavine phosphoric acid, but not by flavine (Vitamin B_2) itself ; conversely, the cortical hormone is ineffective if vitamin B_2 is absent from the diet. The central point in the effects of adrenalectomy is thus claimed to be the failure of the body to synthesise the important flavine phosphate, which is a component of the yellow respiratory enzyme (p. 105). These claims have, however, not been confirmed by subsequent investigators.*

Units. Various units of activity of the cortical principles have been proposed. Thus, the Pfiffner " dog unit " is the minimal daily dose per kg. body weight which will keep an adrenalectomised dog constant in body weight and with a constant low blood urea. If 0·3 g. kg./day of NaCl is given with the diet, the unit is equal to 0·5–1·0 g. of fresh gland, or 0·015–0·020 mg. desoxycorticosterone kg./day. If sodium salts are not administered, at least ten times as much will be needed.

The suprarenal cortex also has a high content of ascorbic acid, which is probably due to its storage there.

FUNCTIONS OF THE MEDULLA

The medulla can be destroyed by operation, or by insertion of a small tube of radium, without causing death. It seems that the functions of the medulla, if essential to life, can be more or less effectively maintained by

* Bruce and Wien. *J. Physiol.*, 1940, **98**, 375.

the other chromaffine tissue found at the back of the abdomen. But there is no conclusive evidence that the chromaffine material is, in fact, essential.

Schafer and Oliver in 1894* found that an aqueous extract of the suprarenal bodies, when injected into the circulation, caused a great rise of blood pressure, brought about chiefly by generalised vaso-constriction. The substance responsible for this was limited to the medulla, and later on, Abel † studied, and Takamine ‡ isolated, the active substance, to which the name of *adrenaline* § was given. Adrenaline gives a blue colour with phosphotungstic acid,|| or with arsenomolybdic acid ; ¶ the latter being especially delicate can be employed for its quantitative estimation. The red colour given with potassium persulphate,** and also other reactions†† can likewise be used for its estimation, but it is more often assayed in biological fluids by a biological

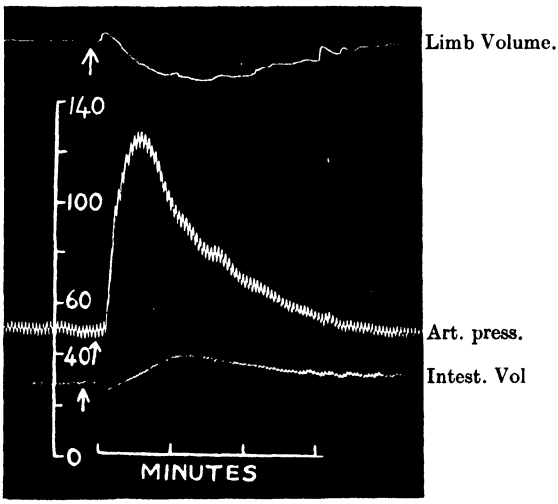
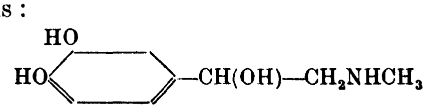


FIG. 617. Effect of Intravenous Injection of Adrenaline on Limb Volume, Arterial Pressure and Intestine Volume of Decerebrate Cat. At arrow 0·01 mg. adrenaline ; passive dilatation of intestine. (G. A. CLARK. *J. Physiol.*, 1930, **69**, 181.)

method, *e.g.*, by the relaxation of a loop of isolated intestine. The constitution of adrenaline is :



The natural form is lævorotatory and is physiologically about twelve times as active as is the dextro-variety.‡‡ Both dextro- and lævo-forms have been prepared synthetically.

It usually is accepted that adrenaline, as prepared from the suprarenals, is identical with the active hormone which these organs can release into the blood stream, and with the synthetic compound.

* SCHAFER and OLIVER. *J. Physiol.*, 1895, **18**, 230.
† ABEL. *Amer. J. Physiol.*, 1900, **3**,
‡ TAKAMINE. *J. Physiol.*, 1901, **27**, xxix.
§ Also called adrenine or epinephrine.
|| BAKER and MARRIAN. *Biochem. Journ.*, 1927, **21**, 1005.
¶ SHAW. *Bioch. J.*, 1938, **32**, 19.
** BARKER *et al.* *Bioch. J.*, 1933, **26**, 2129.
†† WHITEHORN *J. Biol. Chem.*, 1935, **108**, 633. RICHTER *J. Physiol.*, 1940, **98**, 367
‡‡ CUSHNY. *J. Physiol.*, 1908, **38**, 259

Adrenaline, though almost inert when given by mouth, is active in minute amounts on injection into the general circulation, injection of 0·0025 mg. per kg. body weight sufficing to evoke an effect upon blood pressure. On injecting it into the circulation there is immediately a rise of blood pressure which, if the vagi are intact, is only moderate in amount, since it is accompanied by a slowing of the heart. This latter is due to an excitation of the vagus secondary to the rise of blood pressure, and is not due to direct action of the drug on the vagus centre except that it increases its reflex excitability.* If the vagi be divided, the injection of adrenaline, even after destruction of the central nervous system, evokes a huge rise of pressure, which may amount to 300 mm. Hg. The most intensely constricted vessels are those of the skin; in the splanchinc area the effect is smaller, so that if the rise of pressure is large, these vessels may become passively dilated (Fig. 617).

It has been shown by Langley † and by Elliott ‡ that adrenaline injected into the circulation arouses those activities which can normally be excited by stimulation of the sympathetic system. Thus, the liver glycogen is rapidly discharged, and hyperglycæmia and glycosuria result.§ The glycogen of muscle is also discharged, being converted into lactic acid.|| Fatigue of skeletal muscle is postponed. The heat production and oxygen usage are increased.¶ In the head, it causes dilatation of the pupil, secretion of saliva, and erection of the hairs. The dilatation of the pupil takes place much more readily after previous removal of its sympathetic supply by excision of the superior cervical ganglion. Such a 'denervated' pupil will respond to a dose of adrenaline which is much too small to affect the normal one.** On the heart it has a strong augmentor and accelerator influence, greatly increasing its oxygen utilisation,†† so that it beats more effectively, and empties itself more completely in systole, even against the enormously increased resistance offered by the constricted arterioles. The bronchi and bronchioles are fully relaxed ‡‡; respiratory movements are often temporarily inhibited (adrenaline apnœa: this is a secondary effect of the rise of arterial pressure on the carotid sinus). On the lung vessels, adrenaline has probably a slight constrictor influence. With regard to the vessels of the brain, we find the same slight constriction as in the case of excitation of the sympathetic nerves to this organ. In the abdomen, it causes complete relaxation of the musculature of the cat's and the human uterus, of the stomach, and of the small and large intestines, but contraction of the ileocolic sphincter, and of the rabbit's uterus. On the bladder, its effect varies according to the animal studied, but in every case is identical with that obtained by stimulating the hypogastric nerves. It has been shown that very small doses of adrenaline usually cause a definite *fall* of blood pressure in anæsthetised animals. This is probably due to relaxation of the capillaries,§§ particularly in muscle,|||| outweighing the vasoconstrictor effect elsewhere.

* Stella. *J. Physiol.*, 1932, **77**, 68.
† Langley. *J. Physiol.*, 1901, **27**, 237.
‡ Elliott. *J. Physiol.*, 1905, **32**, 401.
§ Blum. *Pflüger's Arch.*, 1902, **90**, 617; Hester and Wakeman *Arch. f. exp. Path.*, 1902, **169**, 478.
|| Cori and Cori. *J. Biol. Chem.*, 1928, **79**, 309.
¶ Boothby and Sandiford. *Amer. J. Physiol.*, 1919, **51**, 200.
** Meltzer and Auer. *Amer. J. Physiol.*, 1904, **11**, 28.
†† Lovatt Evans and Ogawa. *J. Physiol.*, 1914, **47**, 446.
‡‡ Thornton. *Q. J. Exp. Phys.*, 1932, **21**, 305.
§§ Dale and Richards. *J. Physiol.*, 1918, **52**, 110.
|||| Clark. *J. Physiol.*, 1934, **80**, 429.

The motor effects of adrenaline are usually reversed after previous injection of ergotoxine or of ergotamine, the active alkalids of ergot. These drugs, when injected, cause, first, active vasoconstriction, followed by paralysis of the vasoconstrictor mechanism. Excitation of the splanchnic nerves, or injection of adrenaline, will now bring about a fall of blood pressure, due to dilatation of the vessels in the splanchnic area.*

The substance called 933 F. (piperidomethylbenzodioxane) is also an anti-sympathomimetic substance, which reverses the hypertension caused by adrenaline, though, unlike ergotoxine, it does not paralyse adrenergic nerves.

The point of attack of the adrenaline appears to be in the muscular or glandular tissues themselves, since its effects may not only be obtained after destruction of the sympathetic nerves, but even obtained (and in an exaggerated degree) after time has been allowed for the peripheral (post-ganglionic) fibres to degenerate as a result of extirpation of the corresponding ganglia. We have already dealt (p. 369) with the probability that the 'adrenergic' nerves produce their effects by the liberation, at their endings, of adrenaline, or of some related substance.

The splanchnic nerve is the secretory nerve to the suprarenal medulla, which receives a number of filaments from the splanchnic on its own side. These end without the interposition of any ganglion cells on their course,† the cells of the medulla having themselves been developed by a modification of sympathetic ganglion cells. Stimulation of the peripheral end of the splanchnic nerve causes, as we have already seen, a discharge of adrenaline into the blood stream. This discharge accounts for the secondary rise, often accompanied with quickening of the heart, observed on a blood-pressure tracing as the result of stimulating the splanchnic nerve.‡ Through the splanchnic nerves, a discharge of adrenaline can be excited by many general conditions, such as anoxæmia,§ pressure on the brain, puncture of the fourth ventricle,|| administration of anæsthetics, or mental disturbances such as excitement or fright. Such a discharge is an important element in the adaptation to environmental stress, and enables the animal to react for the preservation of its life, either by offence or by flight. If one splanchnic nerve be cut before the administration of anæsthetics or the induction of a condition of irritation or fright, the suprarenal gland on the corresponding side will be found to contain two or three times as much adrenaline as the gland which has been left in connection with the central nervous system. It is stated that no such condition of exhaustion can be produced by electrical stimulation of the peripheral end of the divided splanchnic. This may be because the splanchnic fibres are not very suitably excited by faradic stimulation, and so the recuperative process in the medulla is able to keep pace with the depletion.

Cannon and his co-workers¶ demonstrated the liberation of adrenaline from the suprarenals, by observing the acceleration of the denervated heart. Cats were used in these experiments, and the heart was denervated by removal of the stellate and other thoracic sympathetic ganglia and section of the vagi in the neck. Such animals in states of asphyxia, fear, pain or rage showed pronounced cardiac acceleration, but if the splanchnics were divided, the effect was very slight or absent. Reflex secretion of adrenaline could also be shown in this preparation by stimulation of the central end of the sciatic nerve.

* Dale. *J. Physiol.*, 1913, **46**, 291.
† Elliott. *J. Physiol.*, 1913, **46**, 285.
‡ Anrep. *J. Physiol.*, 1912, **45**, 307.
§ Kellaway. *J. Physiol.*, 1919, **53**, 211.
|| Carrasco-Formiguera. *Amer. J. Physiol.*, 1922, **61**, 254.
¶ Cannon *et al.* *Amer. J. Physiol.* 1921, **58**, 308, 338; 1924, **69**, 46.

Similarly, Kellaway, using a cat with the pupil of one eye deprived of its sympathetic supply, showed that the hypersensitive denervated pupil dilates in even mild degrees of asphyxia or oxygen lack.

Tournade and Chabrol * have also shown that the secretion of adrenaline may be produced reflexly. Their method is to anastomose the suprarenal vein of one dog A, with the external jugular vein of a second dog B. The arterial pressure of B is recorded and the spleen of B placed in a plethysmograph. When the central end of the sciatic nerve of A was stimulated, there was a rise of blood pressure and a shrinkage of the spleen in B. By a similar technique, Heymans † has shown that a fall of blood pressure in the arteries (but especially in the *sinus caroticus*) leads to a reflex liberation of adrenaline. The arrangement of this experiment is shown in Fig. 618. Adrenaline is liberated from the suprarenals of dog B when the carotid pressure on the left (innervated) side is lowered, but not when the carotid artery pressure on the right side is lowered (carotid sinus

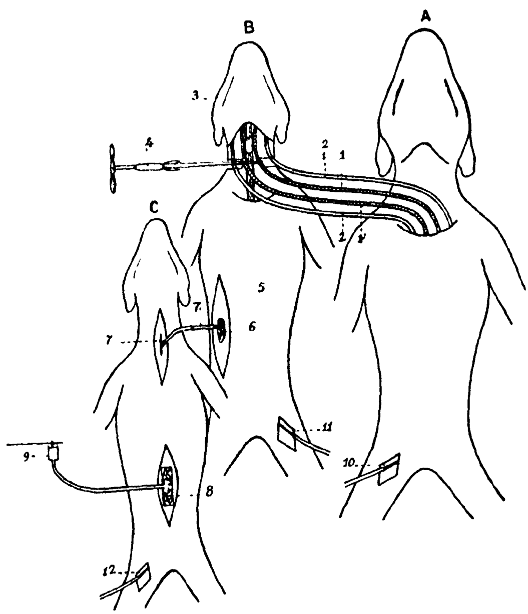


FIG. 618. Perfusion by Carotid and Jugular Anastomosis from A to Semi-isolated Head of B (right carotid sinus denervated), and from Suprarenal Vein of B to Jugular Vein of C (epinephrectomised). Spleen of C in a plethysmograph contracts when adrenaline is liberated by B, as *e.g.* when pressure in left carotid of B is lowered. Systemic pressures recorded from femoral arteries. Vagi of B divided; also all neck tissues divided by *écraseur* (4) except spinal cord. (C. HEYMANS.)

denervated on right side of B). When the adrenaline is liberated in B, the spleen of C (suprarenals removed) contracts.

Stewart and Rogoff, ‡ using other methods, claim to have demonstrated that adrenaline is continually being passed out from the suprarenals into the blood, but it is doubtful whether in normal quietly resting animals the concentration of adrenaline in the blood exceed 1 part in 1,000 millions, and this would produce no effects.

When adrenaline is injected into the blood stream, the effect is only transitory—a matter of seconds for a small dose, *e.g.* 0·01 mg. for a cat. It rapidly disappears from the blood, since it is easily oxidised *in vitro* in alkaline solution, particularly by various enzymes, such as amine oxidases.

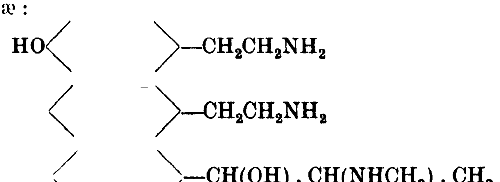
* TOURNADE and CHABROL. *C. R. Soc. biol.*, 1926, **94**, 1199.
† HEYMANS. *Arch. intern. pharmacodyn*, 1929, **35**, 269.
‡ STEWART and ROGOFF. *Amer. J Physiol.*, 1917, **44**, 543 1920, **51**, 366; 1923, **66** 235.

It has been supposed that, after performing its excitatory function, it is destroyed by oxidation in the tissue fluids. It has been shown, however (at all events when given by mouth) to be more probably eliminated by conversion into an ester.* It is partly on this account that when injected into the portal vein adrenaline is relatively ineffective, since the liver is known readily to form sulphate esters with phenolic bodies. Adrenaline is thus a typical hormone, a body of comparatively low molecular weight, having a drug-like excitatory action on specific tissues of the body, easily diffusible, and rapidly destroyed after discharging its office.

There seems little doubt that we must ascribe to the chromaffine tissue the function of elaborating this substance, and it has been claimed † that living medullary tissue can convert *p*-hydroxyphenylethylamine into adrenaline.

What part adrenaline plays in the normal functions of the body has, however, been contested with no little heat. Whether it is continuously discharged into the circulating blood, and, if so, in what amounts and with what, if any, consequences, and whether or not the rate of liberation is augmented by emotion, asphyxia, splanchnic stimulation, fall of blood-sugar, &c., have been the principal subjects of controversy. The bulk of opinion seems to be that adrenaline is very slowly poured out by the normal suprarenal, and that this output is stopped or greatly reduced by section of the splanchnic nerve supply ; that the amount of adrenaline so liberated is too small to exert an effect upon the tone of the arterioles, though it might affect the tone and permeability of the capillaries ; and that, in states of emotion, asphyxia, exposure to cold, &c., there is a sudden liberation of adrenaline in such amount (*e.g.* 0·004 mg./kg./min.) as suffices to produce the effects seen on injection of that substance into the blood stream. According to this view, the *emergency theory*, we should be inclined to regard the suprarenal medulla as resembling the sympathetic nervous system in being a useful adjunct in times of stress, though not essential to life.

Owing to the rapid destruction of adrenaline, it produces much less effect when given by the mouth. There is, however, a whole series of substances, more or less allied to adrenaline in chemical constitution, which undergo less rapid destruction and can therefore be administered by mouth in the usual way. Dale and Barger described such substances produced from the amino-acids of proteins by a process of decarboxylation. Tyrosine would yield hydroxyphenylamine, and phenylalanine would give phenylethylamine Such substances may be formed in minute quantities during the normal processes of putrefaction which occur in the alimentary canal. Another similar substance is the drug ephedrine. The constitution of these substances is shown in the following formulæ :



p-hydroxyphenylethylamine

phenylethylamine

ephedrine

Ephedrine sensitises tissues not only to adrenaline, but also to the stimulation of adrenergic nerves. This appears to be because it inhibits the enzyme, amine oxidase, by which adrenaline is destroyed. ‡ This recalls the action of eserine in potentiating the action of acetyl choline or of cholinergic nerves.

* Richter. *J. Physiol*, 1940, **98**, 361.
† Schuler and Wiedemann. *Z. f. p. C.*, 1935, **233**, 235.
‡ Gaddum and Kwiatkowski. *J. Physiol.*, 1938, **94**, 87.

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THE THYROID

The thyroid gland consists of two oval bodies weighing, in man, 20 to 25 gm., lying on either side of the trachea, and joined, in many animals, across the trachea by an isthmus. Surrounded by a capsule of connective tissue, it is made up of an aggregation of vesicles varying in diameter from 15 to 150μ. The vesicles are lined by a single layer of cubical epithelial cells, and are filled with a translucent material known as colloid (Fig. 621, A). Mitochondria are sometimes very abundant in the cells, and these and the Golgi apparatus undergo definite changes under conditions which are believed to be associated with alterations in the secretory activity of the thyroid, *e.g.* an increase when the animal is exposed to cold. Between the vesicles may be seen, here and there, solid masses of cells which by some observers are regarded as destined to replace vesicles the epithelium of which has undergone complete degeneration. The internal secretion probably makes its way into the blood direct, or along the lymphatic efferents of the gland. The thyroid is richly supplied with blood by three arteries, and is surrounded with a plexus of veins lying immediately under the capsule. It receives at least three times its own weight of blood per minute. The nerve supply is from the cervical sympathetic. In development, the thyroid is formed by a down-growth from the floor of the pharynx.

Diseases due to both deficient and excessive activity of the thyroid are known, and clinical observations of these first gave the clue to the functions of the thyroid.

Thyroid Deficiency. The effects of thyroid deficiency differ according to whether the deficiency is manifested in infancy or in later life.

If the atrophy of the thyroid occurs in early life, during the period of growth, *e.g.* in young children, the growth of the skeleton practically ceases. The bones of the limbs may grow in thickness but not in length. There is early synostosis of the bones of the skull, and complete cessation of development of mental powers. Children so affected are called *Cretins*. They may live for many years, but when twenty-five or thirty still present a childish appearance (Fig. 619, C). Stunted, pot-bellied and ugly, they have the intelligence of children of four or five. They often present fatty tumours above each clavicle, and similar subcutaneous tumours of fat or loose fibrous tissue are found in cases of myxœdema in the adult. The basal metabolism is low, and sugar tolerance high.

Removal of the thyroid from tadpoles causes a comparable failure of proper development, so that metamorphosis is absent or delayed.

If care be taken to spare the parathyroids at the operation, removal of the thyroid from young animals, as Sutherland Simpson showed, results in the same retarded development and stunting of growth as are observed in human beings when there is atrophy of the thyroid gland alone.

In this country, attention was called to the connection of a disturbed condition of metabolism known as *myxœdema* with alteration or atrophy of the thyroid occurring later in life. A patient affected with myxœdema presents a gradually increasing blunting of his or her mental activities ; speech

is slow, cerebration delayed. With this nervous defect are associated changes in the connective tissues, the subcutaneous connective tissue becoming thickened, so that the face and hands appear swollen and puffy, looking at first sight as if œdema were present. The swelling is, however, due to newly-formed connective tissue, and not to the presence of an excess of interstitial fluid in the tissues. The patient often has a yellow waxy appearance, with a patch of colour on the cheeks. The hair falls out, the pulse is slowed, and the temperature tends to be subnormal owing to the diminution of the general metabolism of the body. Determination of the patient's basal metabolic rate shows this to be definitely (20–30 per cent.) below the normal value, as calculated from weight and height.* The intake of food and the excretion

A B C

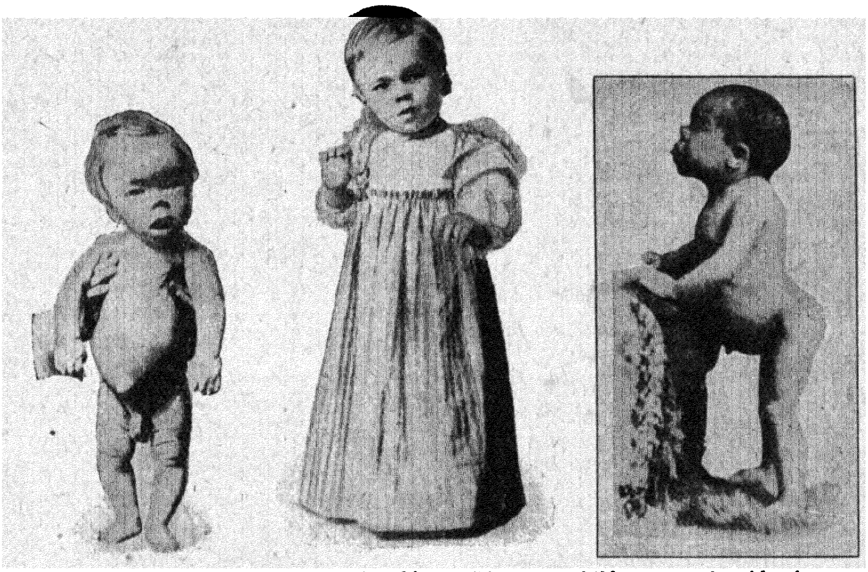


Fig. 619. A. A cretin, 23 months old. B. The same child, 34 months old, after administration of sheep's thyroids for 11 months. C. A cretin, untreated, 15 years old. (W. Osler.)

of nitrogen are diminished. When the thyroid is extirpated in adults the result is often the production of typical myxœdema.

Thyroid Feeding. Schiff first showed that the effects of extirpation of the thyroid might be prevented if, at the same time, the thyroid from another animal were transplanted into the subcutaneous tissue. On removing the transplanted thyroid, the typical symptoms of thyroid deprivation at once ensued. It was later found that it was sufficient to administer the thyroid gland, either fresh, dried, or partially cooked, by the mouth. An ugly and idiotic cretin can be converted by this means into a child of ordinary intelligence with normal powers of growth (Fig. 619, A and B). Given to myxœdematous patients, fresh or dried thyroid gland restores the basal metabolism, nitrogen output and body temperature to their normal levels, reduces the swelling of the subcutaneous tissues, causes a new growth of hair, and reinstates the patient's former condition of mental health †‡

* Magnus-Levy. *Berl. klin. Woch.*, 1895, **32**, 650.
† Murray. *B.M.J.*, 1891 (ii.), 796.
‡ Fox. *B.M.J.*, 1892 (ii.), 941.

(Fig. 620). It is possible that a moderate degree of thyroid inadequacy is not infrequent, and that the beneficial effects on general health, in removing excessive corpulence and in promoting the growth of hair, which are observed on administering thyroid to people in middle life, may be due to the actual reinforcement of a function which is being insufficiently discharged. Nor is the thyroid gland without influence on the healthy individual. When tadpoles are fed with thyroid substance, the rate of metamorphosis is greatly

Fig. 620. Myxœdema treated for thirty years with Thyroid. A, aged 65, before treatment. B, five weeks later. C, fifteen months later. D, aged 94. (After Raven. *B.M.J.*, October 4th, 1924.)

accelerated.* If given in large doses either to man or to animals, it quickens the pulse, even causing violent palpitation, and increases the metabolic activities of the body,† so that the appetite is increased, the glycogen store of the liver is reduced, the nitrogenous output rises above the intake, and the subcutaneous fat is diminished or disappears.

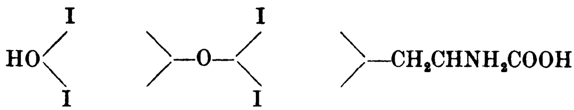
There are grounds for the belief that the thyroid plays a part in the production of the increased metabolism and rise of temperature in fevers

* Gudernatsch. *Amer. J. Anat.*, 1914, **15**, 431.

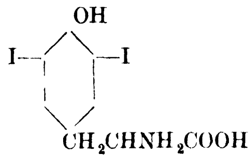
† Anderson and Bergmann. *Skand. Arch. f. Physiol.*, 1898, **8**, 326.

and it has also been claimed that the specific dynamic action of proteins is dependent on the presence of the thyroid.* It may also, by producing its secretion more rapidly, be concerned in causing the rise of metabolism which results when there is a tendency for the body temperature to be lowered ; under these conditions the blood flow through the thyroid is increased,† and the gland shows histological changes.

Thyroxine. The specific material of the colloid, called *thyroglobulin*, contains iodine in organic combination. Kendall, in 1914, isolated an active principle by hydrolysis of the thyroid, and gave it the name *thyroxine*. Harington and Barger in 1927‡ showed this to be the *l*-compound, and to have the structural formula :



and prepared the compound synthetically. About 60 per cent. of the iodine of the thyroid is present in another and simpler compound, *di-iodotyrosine*, which is physiologically much less active :—



(Di-iodotyrosine)

By treating casein with iodine it has been shown that thyroxin can be obtained in small amounts.§

The actual hormone produced by the gland is probably a peptide containing both these iodine-containing amino acids, together perhaps with other amino-acids. Its activity is greater than that of its contained individual amino acids.||

The thyroid of an average human adult probably contains about 20 mg. of thyroxine. This substance, when administered, exhibits all the therapeutic properties of the dried gland itself. Thus 1 mg. of thyroxine definitely raises the basal metabolic rate, the maximal effect appearing after a day or two. It also relieves the condition of hypothyroidism, and accelerates the metamorphosis of tadpoles. It is believed that the thyroid of the normal individual produces about 1 mg. thyroxine a day.

The thyroxine is presumably manufactured in the thyroid itself, presumably from tyrosine, *via* iodotyrosine. The gland has a remarkable affinity for inorganic iodine ; usually it stores about 2 mg. per gram of dry gland in man, but in most mammals there is about 5 mg. If iodide be injected into the circulation, the greater part of it may be recovered from the thyroid within five minutes.

Although thyroxine produces effects so similar to those of the gland itself, the evidence that thyroxine, as such, is eliminated by the thyroid, or circulates in the blood is equivocal. Normal blood, however, does contain small amounts of iodine, about 3 μg. per 100 c.c., and this may be increased from two- to

* BAUMANN and HUNT. *J. Biol. Chem.*, 1925, **64**, 709.

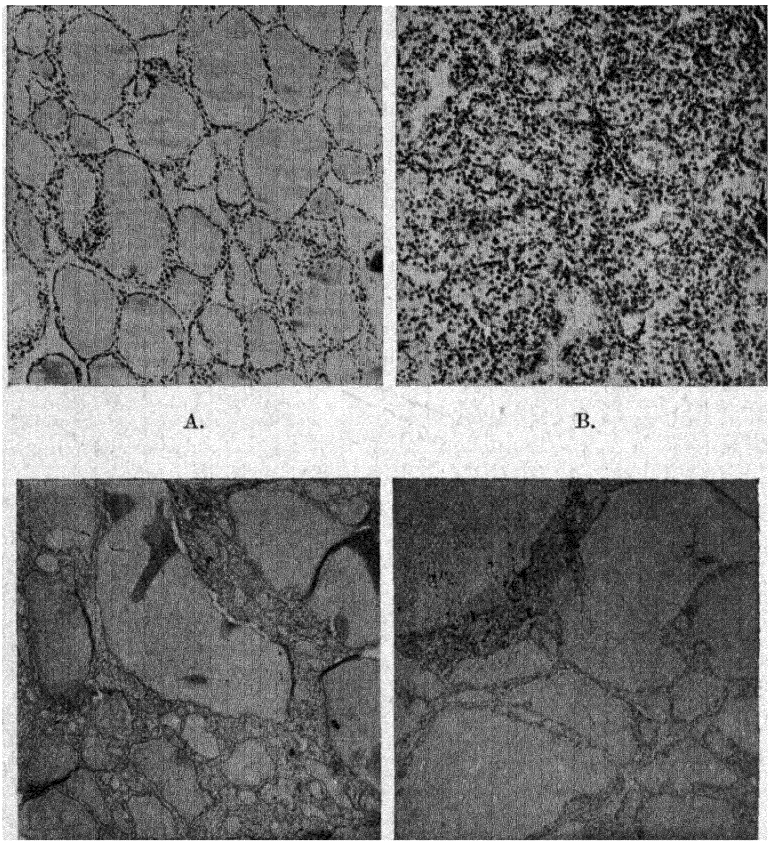
† DIETRICH and SCHWIEGK. *Arch. f. exp. Path. u. Pharm.*, 1932, **165**, 53.

‡ HARINGTON and BARGER. *Bioch. Journ.*, 1927, **21**, 169.

§ HARINGTON and PITT-RIVERS. *Nature*, 1939, **144**, 205.

|| HARINGTON. *B.M.J.*, 1936 (ii.), 1269.

five-fold in exophthalmic goitre By the intravenous injection of iodine the iodine content of the blood is temporarily raised, but falls to normal in an hour or two, most rapidly in hyperthyroid subjects and most slowly in the hypothyroid ones.* The delay which elapses between the administration of thyroxine, and the production of physiological effects, may mean that the relatively simple molecule of that substance needs to be built up into



C D.

FIG 621. Illustrating the Influence of Iodine Lack on Thyroid Structure A = thyroid of puppy on diet which included cod liver oil (containing iodine)—normal structure B = thyroid of puppy on same diet except that it contained peanut oil (no iodine)—hyperplasia C = human thyroid, simple colloid goitre. D = simple colloid goitre in dog's thyroid. the dog was iodine deficient for 10 months, after which KI was given daily for 8 months (15γI daily.) (E MELLANBY. "Nutrition and Disease." Oliver and Boyd, 1934.)

some more elaborate molecule before it can be effective. The earliest active materials prepared from the thyroid were, in fact, globulins, of which thyroxine formed one of the constituents.

Harington † suggests that the active principle contains both thyroxine and di-iodotyrosine, linked by a chain of other amino-acids to form a poly-

* WATSON *Endocrinol*, 1938, **22**, 528.
† HARINGTON *Lancet*, 1935, i, 1261.

peptide, which is built up into the thyroglobulin molecule. Presumably di-iodotyrosine is also a first step towards this synthesis.

Thyroxine produces no effects when added to isolated tissues, although tissues taken from animals previously dosed with the active principles show an increased rate of oxygen usage. Thyroglobulin does cause an increase in the oxygen usage of isolated tissues,* from which it may be inferred that the delay in the action of thyroxine is due to the time needed for its conversion into thyroglobulin.

IODINE LACK. If iodine be entirely absent from the drinking water and food, the thyroid undergoes enlargement; this is most clearly seen if the deprivation occurs during ante-natal life by reason of the mother having been fed on foods lacking in iodine before and during pregnancy. The resulting enlarged thyroid of the offspring has the structure of a parenchymatous hyperplastic goitre. The glandular tissue is increased in amount and the colloid reduced or absent (Fig. 621, B). If the lack of iodine is continued after birth the gland undergoes further hyperplasia. Early administration of small amounts of iodine in the form of iodide restores the gland to its normal size and structure, but if given later on in life complete restoration is unlikely, the gland instead undergoing further enlargement and showing on histological examination (Fig. 621, D) large irregular vesicles distended with colloid—the 'colloid goitre.'† Very large doses, however, may be more effective. Of common foods, fish is richest in iodine. Absence of iodine from the soil and water seems to be the cause of the great prevalence of goitre in certain inland districts, especially in Switzerland and in parts of the United States. It has been shown by Marine and others that this endemic goitre can be practically eliminated from these districts by the occasional administration of minute doses of iodine or iodides to the entire population (as by addition to the water supply or to the table salt).

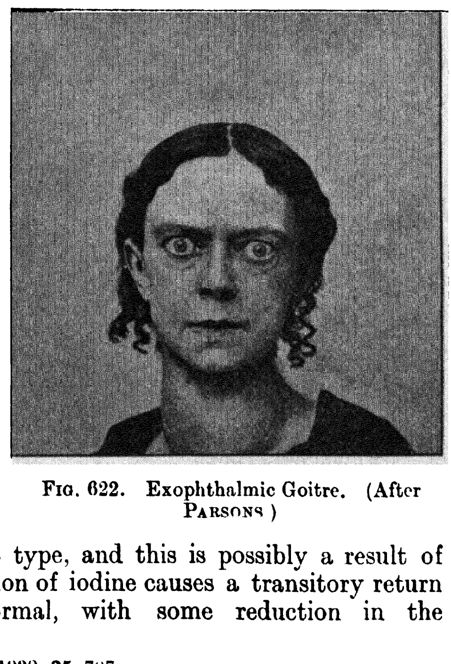


FIG. 622. Exophthalmic Goitre. (After PARSONS.)

Hyperthyroidism. The symptoms caused by excessive doses of thyroid gland are strikingly similar to those occurring in the disease known as *exophthalmic goitre* (Fig. 622), where there is a true hypertrophy of the gland associated with increased basal metabolic rate, a body temperature tending to be above the normal, cardiac palpitation, proptosis (bulging of the eyes), wasting, great restlessness, occasional glycosuria, and muscular weakness. The iodine content of the blood is usually raised, while the thyroid itself contains less iodine than normally. Its structure is of the hyperplastic type, and this is possibly a result of its low iodine content. Administration of iodine causes a transitory return of the structure towards the normal, with some reduction in the

* CANZANELLI *et al.* *Endocrinol.*, 1939, **25**, 707

† E. MELLANBY. Nutrition and Disease. (1934: Oliver and Boyd.)

symptoms, but these become worse later on especially if the iodine is discontinued.

In these cases, removal of part of the hypertrophied gland often removes the symptoms and restores the patient to health. In some cases, however, the condition recurs. If the whole gland is removed, it is necessary to administer daily doses of dried gland for the rest of the patient's life, in order to prevent the development of myxœdema.

One view of the condition is that it is due to a failure of the gland to retain the thyroxine which it makes. This is attributed to a hormone present in the anterior lobe of the pituitary body.

Thyrotrophic Hormone. As will be shown later, the pituitary anterior lobe is closely related to the activity of the thyroid. Extracts of the anterior pituitary can be prepared which, on injection, cause hypertrophy and hyperactivity of the thyroid, and even exophthalmos. Injection of such extracts has resulted in increase in basal metabolism, hyperplastic change of structure in the thyroid, and increase of blood iodine.*

Thyroid and Vitamins. In some species (rats), administration of thyroid, or of thyroxin, increases the requirement of vitamin B_1, lack of which has been demonstrated in the tissues. If adequate amounts of the vitamin are given, the loss of weight from thyroid administration is lessened.† The effects of vitamins A ‡ and C is similar, as regards loss of weight.

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THE PARATHYROIDS

The parathyroids, discovered by Sandstrom in 1880, § are four oval bodies, which, in man, together weigh about 140 mg., situated, two on each side, on the border of the thyroid gland or actually embedded in its substance. One of each pair is developed from the 3rd, and one from the 4th branchial pouch. In histological appearance, they consist of solid masses or columns of epithelial cells, most of which are pale and hyaline, but a few of which are oxyphil, surrounded with connective tissue and richly supplied with blood sinusoids (Fig. 623). In some animals, *e.g.* in the dog, they are embedded in the thyroid. In others, such as the rabbit, they lie outside the gland, and it is easy to avoid them in the excision of the thyroid. Accessory parathyroid tissue is also frequently found in the thymus, and along the aortic arch. To this varying distribution of the parathyroids is due the discrepant early results of extirpation of the thyroid in carnivora and herbivora respectively. The parathyroids have often been inadvertently removed in man, with fatal results, in the operation of thyroidectomy, prior to a knowledge of their importance.

In spite of their close anatomical association with the thyroid, the

* KROGH, LINDBERG and OKKELS, SCHITTENHELM and EISLER. *Cit.* E. Mellanby.

† PETERS and ROSSITER. *Bioch. J.*, 1939. **33**, 1140.

‡ LOGARAS and DRUMMOND. *Bioch. J.*, 1938, **32**, 964.

§ UPSALA LÄK. FORH 1880, **15**, 441. [English translation by Seipel, *Bull. Inst. Hist. Med.*, 1938, **6**, 179]

parathyroids have a function entirely distinct from that of this gland. Whereas removal of the thyroid produces simply a condition of myxœdema, or retardation of growth in the young animal, removal of the parathyroids is followed by nervous disturbances and *tetany*, which rapidly prove fatal, especially in young animals.* The motor nerves are inordinately excitable to galvanic currents. In man, tetany consists in a tendency for muscles, especially of the forearms, to undergo painful spasms. There is flexion of the metacarpo-phalangeal joints, the interphalangeal joints being extended, and thumb and little finger are drawn towards the palm. Wrist and elbow may be flexed and forearm slightly pronated. The carpal spasm may be precipitated by pressure on the upper arm with a sphygmomanometer

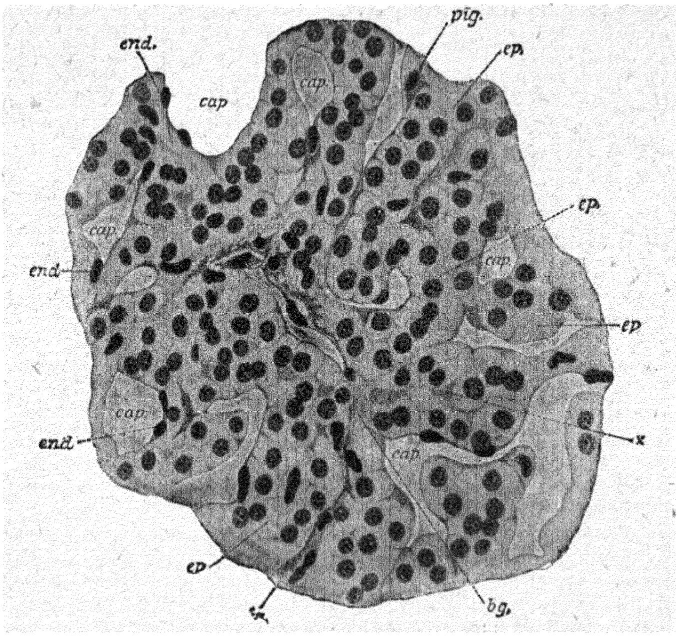

FIG. 623. Section of Parathyroid. (KOHN.)
ep. Secreting epithelium. *cap.* Sinus-like capillaries.
pig. Cells containing pigment. *end.* Endothelial cells.

armlet (Trousseau sign). The condition is always found to be accompanied by a diminution of the calcium content and increase of P content of the blood,† which falls from the normal figure of about 10 mg. Ca per 100 c.c. serum to between 4 and 7 mg. The excretion of calcium and of phosphate is reduced. In chronic cases of hypoparathyroidism defects of skin, hair and nails occur, and cataract of the lens is encountered. The symptoms are found to be temporarily relieved by administration of calcium lactate, either by the mouth or subcutaneously, and are aggravated by a meat diet. The condition of tetany after parathyroidectomy may be relieved by subcutaneous implantation of a normal gland.

Collip‡ discovered that, by extraction of fresh parathyroid glands with 5 per cent. hydrochloric acid and then precipitating the proteins by neutralisation, a fluid is obtained which contains the hormone of the parathyroids in a very

* GLEY. *C. R. Soc. Biol.*, 1897, **4**, 18.
† MACCALLUM and VOEGTLIN. *J. Exp. Med.*, 1909, **11**, 118.
‡ COLLIP. *J. Biol. Chem.*, 1925, **63**, 395; *Amer. J. Physiol.*, 1926, **76**, 472.

potent form. One or two injections per day of this fluid serves to ward off indefinitely the symptoms of tetany in parathyroidectomised dogs, or to cure such symptoms when they have already supervened, either in dogs or in human beings suffering from tetany due to parathyroid insufficiency. The parathyroids are thus the seat of formation of a hormone which regulates calcium metabolism through a specific control of blood calcium concentration.

The active principle is destroyed in the alimentary canal, so that it is ineffective by mouth.

When the extract is administered to normal dogs there is, after a lag of three to four hours, a steady rise in the calcium content of the serum, which reaches a maximum in five to nine hours, and then returns at the same rate to normal. The blood phosphorus is lowered as the calcium rises, except when large doses are given. Excretion of calcium and phosphorus are increased. When several successive injections are made, there is a summation of effect, and the calcium value of the serum may rise to 15 or 20 milligrammes per 100 c.c. When the figure of 15 is passed, the dog shows definite symptoms of 'hypercalcæmia.' The animals lose appetite, become weak, there is diarrhœa and vomiting and rise of blood urea, followed by collapse and death. The impairment of the circulation is associated with great increase in the viscosity of the blood. Prolonged overdosage may lead to the formation of renal calculi, which consist of calcium phosphate, and to calcifications of various tissues.

Parathyroid extracts ("parathormone") are standardised by administration to dogs and determination of the increase in serum calcium in a given time. The unit is defined as 1/500th of the amount required to produce in a dog of 20 kg. weight an increase of 5 mg./100 c.c. in serum calcium in fifteen hours.* The most potent dry preparations contain about 12 units per mg. In man 100 units per day will raise a low blood calcium by 50 per cent., but when the blood calcium is normal even 500 units may only cause a small rise.

Calcium of Blood and Bone. The action of the parathyroid hormone is closely connected with the complex and largely unsolved problems of calcium metabolism. Of the normal serum content of calcium of about 10 mg./100 cc., probably less than half is present in the ionizable form, the remainder being in the form of non-diffusable and unionized complexes of uncertain nature. The ratio between these two forms seems to depend on the *p*H, the protein, the phosphate, and probably other things. Thus, if phosphate is added to the blood, either *in vitro* or *in vivo*, there is a conversion of Ca ions into a physiologically inert and colloidal form, as calcium phosphate.† In the body this colloidal calcium phosphate is phagocytosed by macrophages of the liver and spleen, and so removed from circulation.‡ The excitability of the neuro-muscular mechanisms appears to depend chiefly on the content of ionizable calcium present, and the causation of tetany by rise of *p*H (as by overbreathing) may be ascribed to reduction of the ionic calcium of the blood and tissues.

The principal store of calcium is in the bones. The nature of the mineral basis of bone is still a matter for dispute; it contains calcium phosphate and carbonate, or hydroxide, in some close chemical or physico-chemical union, and appears to be of constant composition.§ The relations of vitamin D to calcium metabolism have been discussed briefly on p. 857, and it was there stated that the calcification of bone is probably dependent upon the liberation of phosphate from organic combination owing to the action of the enzyme phosphatase, which is present in the bone. In this and in many other ways the metabolism of calcium and of phosphate are closely related.

Under the influence of calcium lack, or of the action of the parathyroid hormone, calcium is removed from the bones, especially from the trabeculæ of the cancellous bone. The excretion of calcium and of phosphate in the urine is increased. In lead poisoning, lead is deposited with calcium in the bone, and may remain there for long periods if the calcium is not mobilised; if a dose of parathyroid hormone is given, however, the lead is released with the calcium,‖ and excreted. If a period of rest is now given, the calcium

* Collip's original unit was 1/100th of the amount so required. The fivefold reduction was agreed upon in 1934.

† McLean and Hinrichs. *Amer. J. Physiol.*, 1938, **121**, 580.

‡ Gersh *Ibid.*, p. 589.

§ Kay. *Ann. Rev. Biochem.*, 1932, **1**, 189. Thompson and Collip, *Physiol. Rev.*, 1932, 589, **12**, 340.

‖ Hunter. *Lancet*, 1930, **218**, 897, 947, 999.

is replaced in the bone, and, on giving a second dose of parathyroid, calcium without lead is excreted. It seems that the parathyroid acts by stimulating the formation of new osteoclasts, which exert their solvent action on the calcium salts of the bone. At a later stage of overdosage, however, a reaction may occur, and the animal becomes relatively immune : in this stage osteoblasts are increased and new bone formed rapidly.* It has also been thought that the action of vitamin D may be due to a stimulant action on the parathyroid, but the evidence for this is not free from objections. The whole subject is difficult and extremely complex, and the reader is referred to the reference articles for a fuller discussion.

Hyper- and Hypo-parathyroidism. Occasionally cases of parathyroid adenomata occur, and show all symptoms of hyper-parathyroidism—high Ca and low P in the serum, increased Ca and P excretion, softening or fibrocystic disease of the bones, and often renal phosphatic calculi. Removal of the tumour, which may weigh several grammes, cures the condition. The converse state of hypoparathyroidism, accompanied by low blood Ca, high P, reduced Ca and P excretion, and signs of tetany, may occur spontaneously, or after surgical operations on the thyroid.

Parathyrotrophic Hormone. Extracts of the anterior pituitary can be made which, on injection, lead to hyperplasia of the parathyroids, accompanied by increase of blood calcium and all the symptoms of over-activity of the parathyroids.

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THE PITUITARY BODY (Hypophysis)

In the pituitary body, which weighs about 0·5 g. in man, we have four important structures, the *anterior lobe, posterior lobe, pars intermedia*, and the *pars tuberalis*.† It consists of two main parts, anterior and posterior, which have separate modes of origin. An outgrowth from the buccal cavity in the embryo meets a hollow extension of the anterior cerebral vesicle. The buccal ectoderm gives rise to the anterior lobe and pars intermedia of the pituitary, while the neural epiblast becomes developed into the posterior lobe (Fig. 624). In some animals, the posterior lobe remains hollow and retains its primitive connection with the third ventricle of the brain, but in man it becomes entirely solid.

The anterior lobe in the adult consists of nests of epithelial cells, many of which, the chromophobe cells, are only stained with difficulty, while some (the α-cells) are filled with acidophile and others (the β-cells) with basophile granules. The chromophobe cells are parent cells from which the two types of chromophile cells are formed. In man there are normally about 50 per cent. chromophobe, forty of α- and ten of β-cells.

Striking changes in the histology of the anterior lobe occur under certain conditions. For example, after castration the basophile cells are increased : in pregnancy, cells called pregnancy cells appear ; they are finely granular acidophile cells, derived from chromophobe cells ; after thyroidectomy there is diminution in the acidophiles and vacuolation of basophiles ; in hyperthyroidism the acidophiles increase ; œstrone injections lead to increase, and then to degeneration of the basophiles, later the acidophiles are increased.

The anterior lobe is separated from the posterior lobe by a cleft, which is the remains of the original hollow outgrowth from the buccal cavity. The anterior lobe and pars tuberalis are highly vascular, the other parts only poorly supplied with vessels. The epithelial tissue immediately adjoining the cleft probably belongs to the *pars intermedia* and differs from that constituting the anterior lobe. Its cells, which present but few granules, are arranged in islets, separated by an intervening tissue continuous with the main mass of the posterior lobe. Many of the islets are hollow and enclose a colloid material. The amount of colloid material increases in animals which have undergone extirpation of the thyroid gland.

The colloid material of the anterior lobe has been traced by Herring into the stalk and is believed to contain active materials which ultimately find their way into the

* SELYE. *Endocrinol.*, 1932, 16, 547.

† HERRING. *Q. J. Exp. Physiol.*, 1908, 1, 121, 161, 261 ; 1914, 8, 245, 267.

third ventricle ; it has been supposed that the active constituents of the posterior lobe may also enter the cerebrospinal fluid. An interesting vascular arrangement has been described by Popa and Fielding,* which favours these beliefs. They find that the sinusoids of the anterior part and the capillaries of the posterior lobe unite to form veins which pass up the stalk and then back into a second capillary net in the floor of the third ventricle. In these *hypophysio-portal* vessels globules of colloid material are often visible.

The *posterior lobe* includes the pars nervosa and sometimes the pars intermedia. The chief part, the *pars nervosa*, is composed of neuroglial tissue and spindle-shaped cells called *pituicytes*.†

The *pars tuberalis* surrounds the infundibular stalk, and spreads out on the base of the brain in the neighbourhood of the tuber cinereum. It is histologically quite different from the pars intermedia and anterior lobe. It is much more vascular than the pars intermedia, and has an acinar structure ; the eosinophile cells characteristic of the anterior lobe are wanting. The interest of the pars tuberalis is due to the fact that

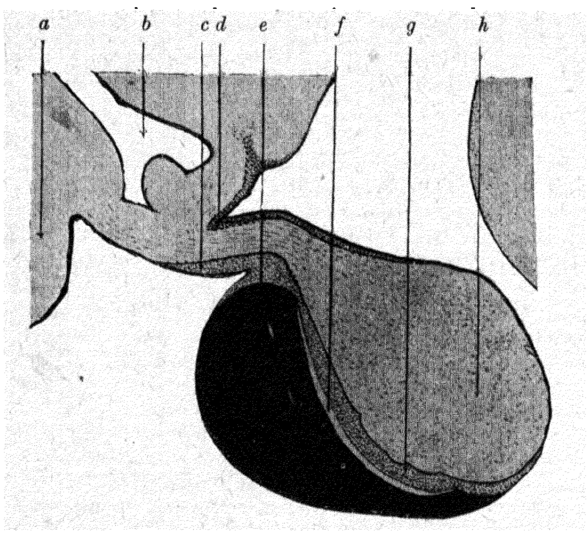

FIG. 624. Mesial Sagittal Section through the Pituitary Body of an Adult Monkey. (Semi-diagrammatic.) (After HERRING.)

a. Optic chiasma.
b. Third ventricle.
c. Pars tuberalis.
d. Epithelial investment of posterior lobe.
e. Anterior lobe.
f. Epithelial cleft.
g. Pars intermedia.
h. Posterior lobe.

it has been found that many of the results attributed to removal of the pituitary body can be obtained by merely injuring the hypothalamic region ; these results are polyuria, glycosuria, loss of heat regulation, and early death.‡ An important feature of the results is atrophy of the posterior lobe. The pituitary receives sympathetic post-ganglionic nerve fibres; their cell stations are in the superior cervical ganglion It is perhaps of importance that there are nervous connections (the supraoptico-hypophyseal and the tubero-hypophyseal tracts, Fig. 635) between the hypothalamus and the pituitary. These are efferent tracts which can be traced to the posterior lobe

Nerve endings are abundant in the posterior lobe and pars intermedia, but in the anterior lobe are chiefly related to the blood vessels.§

The two lobes, anterior and posterior, certainly have entirely different functions. The *pars intermedia* is closely adherent to the posterior lobe, and its functions have not been dissociated from those of the latter.

* POPA and FIELDING. *J Anat.*, 1933, **67**, 227.
† BUCY. *J. Comp. Neurol*, 1930, **50**, 505
‡ CUSHING. *Lancet*, 1930 (ii.), 119, 175
§ RASMUSSEN *Endocrin* , 1938, **23**, 263

THE ANTERIOR LOBE

The anterior lobe is now known to be one of the most important of the endocrine organs, for it produces at least six groups of hormones, several of

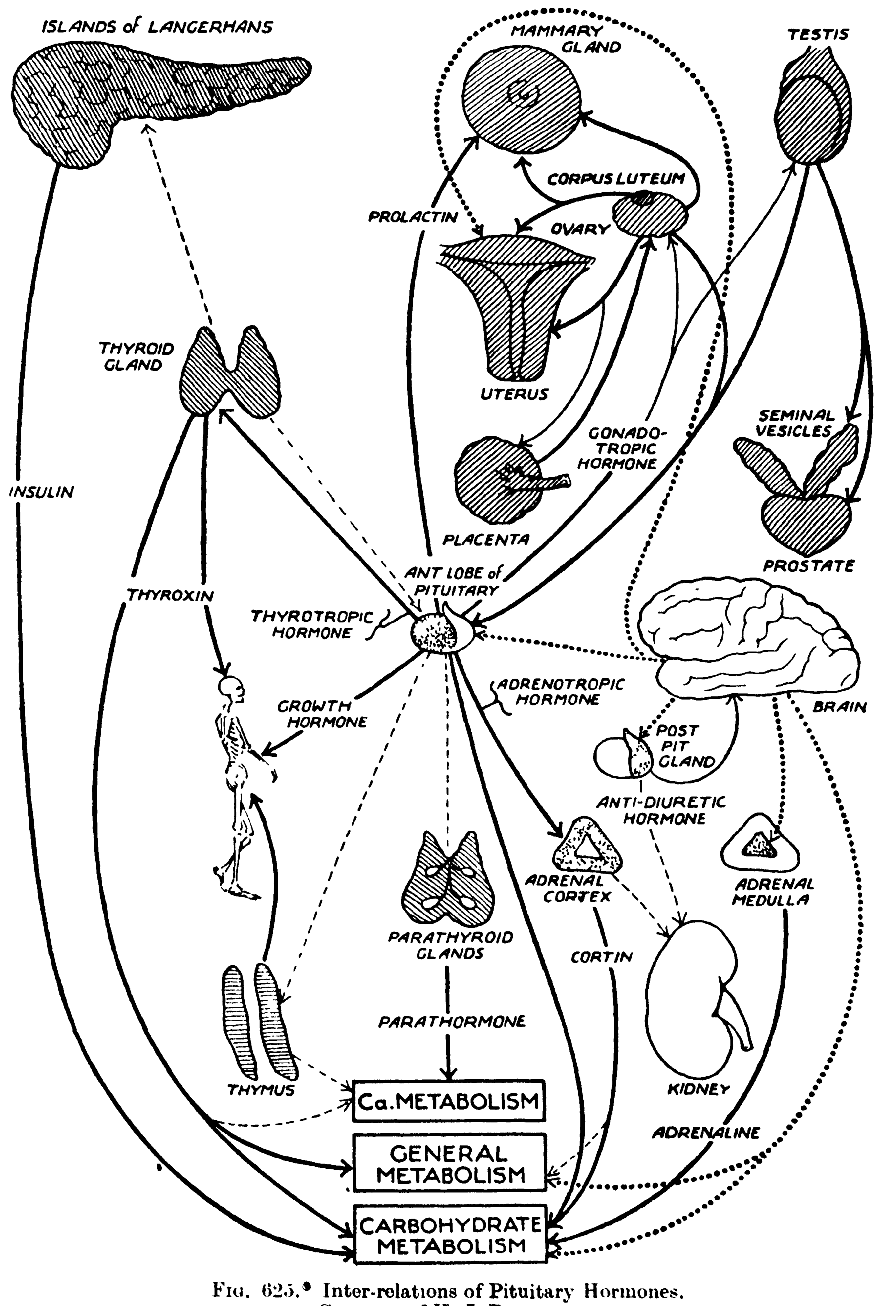

Fig. 625.* Inter-relations of Pituitary Hormones. (Courtesy of H. J. Beattie.)

which have the property of exerting a stimulating action on some other endocrine organ * (Fig. 625). These are

(1) The growth hormones,
(2) The sex or gonadotrophic hormones (follicle-stimulating and luteinizing hormones),
(3) A lactogenic hormone (prolactin),
(4) The thyrotrophic hormone,
(5) An adrenotrophic hormone,
(6) Metabolic hormones, one affecting primarily the metabolism of carbohydrate, and one that of fat.

Parathyrotrophic and pancreatrophic effects have also been described.

It is evident that the presence of so many different hormones, all of obscure chemical nature and none of which have been isolated, makes the study of the anterior pituitary highly interesting but extremely complex. It is still doubtful how many of these separate actions are to be ascribed to chemically distinct individual hormones, but that the description of the anterior pituitary as the master gland of the endocrine system is correct cannot be doubted.

Effects of Extirpation. The effects of extirpation of the whole pituitary (hypophysectomy) are mainly due to the removal of the anterior lobe. Extirpation of this lobe, by abruptly removing the source of all the anterior pituitary " factors " or hormones, as would be expected, produces extensive and complex results. In clinical cases of pituitary hypofunction in which different factors may be affected to different extents, and in which, accordingly, the symptoms are very variable, effects identical with those seen on experimental extirpation are not to be anticipated, and in fact are rarely, if ever, encountered. Our fundamental clues have, however, been gleaned from clinical observation, and confirmed and supplemented by properly controlled laboratory experiments on the lower animals.

The pituitary body is not essential to life, but its extirpation is followed by profound changes.† Growth is arrested sooner or later, there is loss of muscle tone, the reproductive organs fail to mature, or atrophy, there is also atrophy of the thyroid, suprarenal and probably other endocrine organs, a high sugar tolerance, and often abundant deposition of adipose tissue. This latter is, however, stated to be due rather to incidental injury to the hypothalamic region than to the removal of the pituitary itself.

In the human subject, generalised hypofunction of the anterior lobe is found in Simmond's disease (Fig. 626); there is lowered metabolism, cachexia, loss of sexual function and premature senility. In the disease called progeria, which is also probably of pituitary origin (Fig. 627), the senesence is very dramatic. After death atrophy of the anterior lobe is found.

It is interesting to note that there is a reciprocal influence between the anterior pituitary and the various organs which it can affect. Thus, pregnancy, or extirpation of the gonads, thyroid or suprarenals causes definite histological changes to occur in the anterior lobe of the pituitary body.

(1) The Growth Hormone.‡ The first clue to the functions of the anterior pituitary body was furnished by the observations of Marie § that the disease

* SMITH. *J. Amer. Med. Assoc.*, 1935, **104**, 548.
† MCPHAIL. *Proc. Roy. Soc.*, 1935, **117B**, 45.
‡ H. M. EVANS. *J. Amer. Med. Assoc.*, 1935, **104**, 1232.
§ MARIE. *Rev. Med.*, 1886, 298; *Brain*, 1890, **12**, 59.

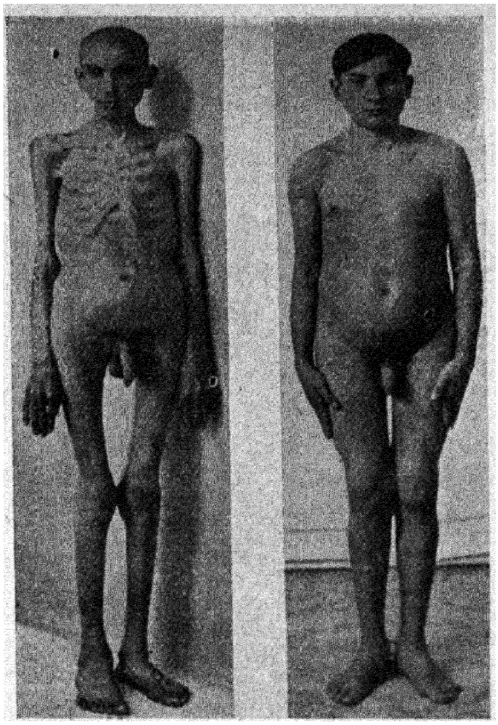

FIG. 626. Simmond's Disease before and after Treatment by Anterior Pituitary Injections. (From LUCACER *La Riforma Med*, 1932, **48**, 547.)

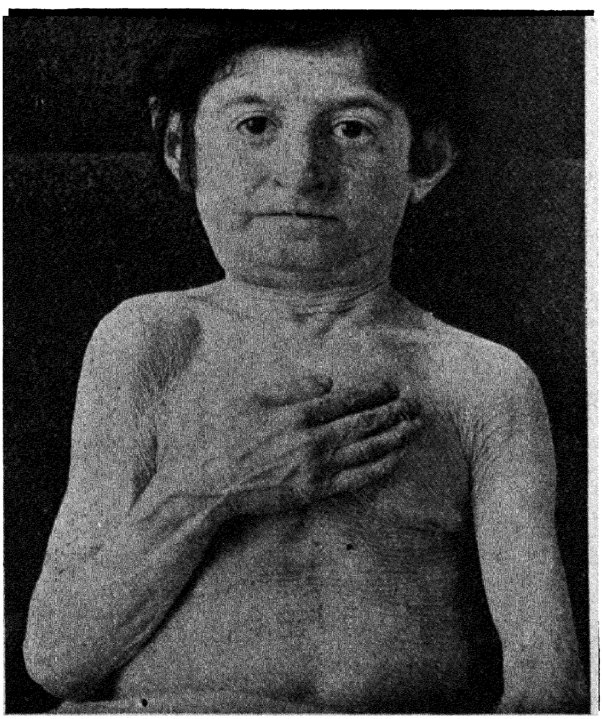

FIG. 627. Progeria in a Girl 8 years of age (From a photograph kindly lent by Professor HARVEY CUSHING.)

called *acromegaly* is associated with pituitary tumour, and it is now known that the tumours consist of the eosinophile cells. In this disease certain parts of the skeleton, particularly of the lower jaw, hands and feet, take on renewed growth in adult life (Fig. 628). Various other symptoms not

FIG. 628. A Case of Acromegaly. I., aged 24, before onset of the disease II, aged 29. III, aged 37. IV., aged 42, with pronounced acromegaly. (After CUSHING. "The Pituitary Body and its Disorders" Lippincott, 1912.)

related to the increase in the production of growth hormone are also often present. Thus there may be limitation of the field of vision, leading to blindness; this begins in the temporal fields owing to pressure of the tumour on the optic nerve fibres from the nasal parts of the retinæ. The

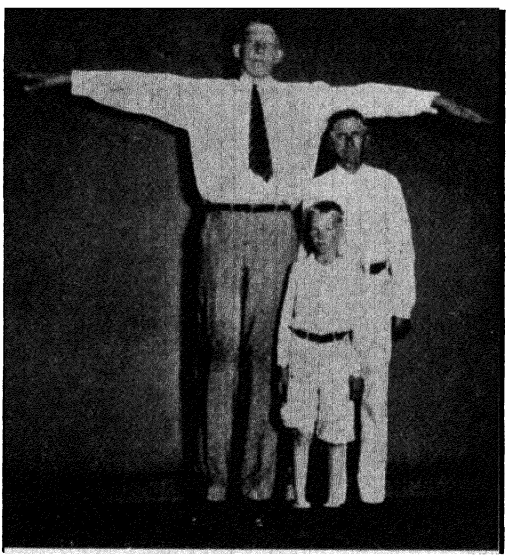

FIG. 629. Giant, aged 13½, beside a 9-year-old Brother, and his Father, who is 5 ft. 11 in. in height. (After BEHIEMS and BARR. *Endocrinology*, 1932, **16**, 125.)

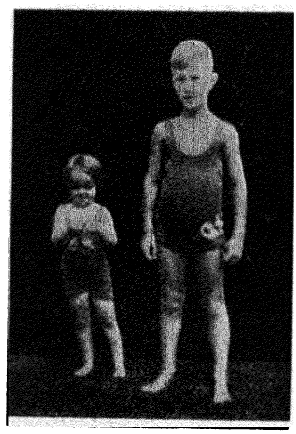

FIG. 631. Female Pituitary Dwarf aged 9½ compared with a Normal Boy of the same age. (After ENGELBACH. *Endocrinology*, 1932, **16**, 11)

condition is relieved by surgical removal of the tumour. There is also not infrequently a lowered sugar tolerance or even diabetes, which may be due to an associated overproduction of other hormones.

GIGANTISM AND DWARFISM. When the disease occurs during the period of active growth, the limbs and trunk bones also grow to an abormal size resulting in a condition of "*gigantism*" (Figs. 629, 632). In such giants, X-ray examination usually shows the presence of an abnormally large pituitary fossa, and there seems little doubt that this condition is due to

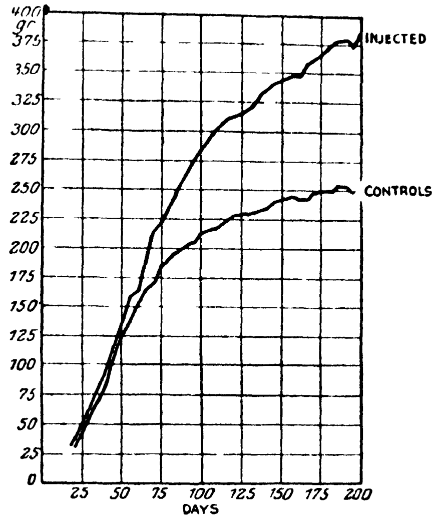

FIG. 630. Effect of Daily Injections of Anterior Pituitary Extracts on the average weights of 38 rats, as compared with 38 untreated controls. (After EVANS and LONG.)

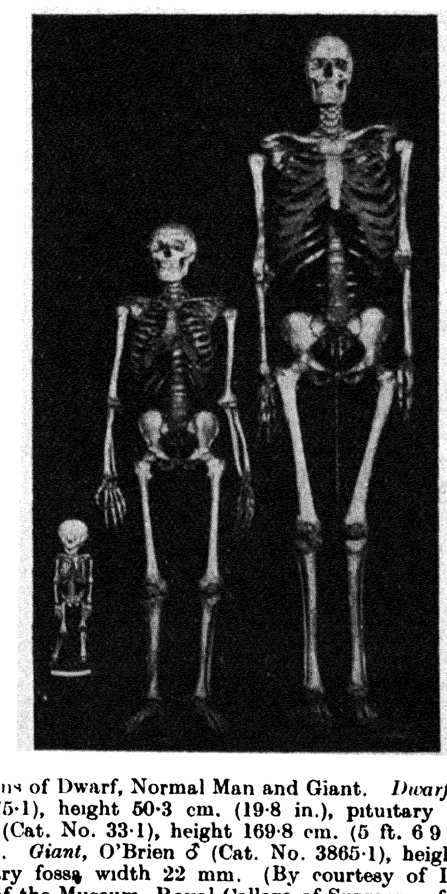

FIG. 632. Skeletons of Dwarf, Normal Man and Giant. *Dwarf*, Caroline Crachami (Cat. No. 4175·1), height 50·3 cm. (19·8 in.), pituitary fossa width 8 mm. *Normal Man* (Cat. No. 33·1), height 169·8 cm. (5 ft. 6·9 in.), pituitary fossa width 15 mm. *Giant*, O'Brien ♂ (Cat. No. 3865·1), height 231·1 cm. (7 ft 7 in.), pituitary fossa width 22 mm. (By courtesy of Dr. H. J. BEATTIE, Conservator of the Museum, Royal College of Surgeons of England.)

an over-action by which the growth hormone is produced at an abnormally high rate.

Experimental gigantism (*e.g.* rats 50 per cent. above the normal size, and of twice the normal weight) has been produced in animals by the injection of partially purified growth hormone, prepared from extracts of the anterior lobe * (Fig. 630). Skeletal growth, checked by hypophysectomy, is restarted by the hormone, and it has also been found that feeding of amphibian larvæ with fresh anterior pituitary results in accelerated metamorphosis. The growth hormone also has an effect on protein metabolism, which consists of an increased laying down of protein in muscles and a diminution of exogenous protein breakdown. Muscular tone and power are augmented by the hormone. The growth hormone may be inactivated in pituitary extracts by adding small amounts of tricresol.

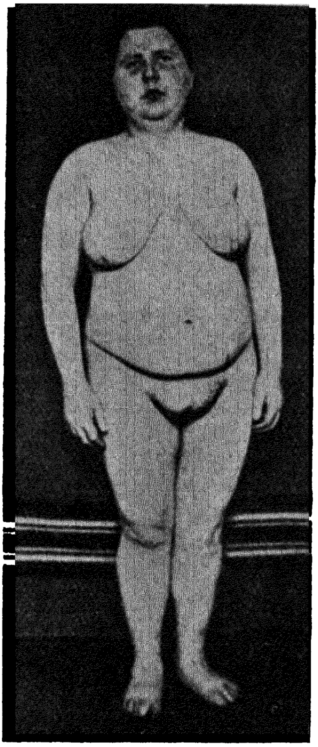

FIG 633 Frohlich's Syndrome. (From DEUSCH. *Deutrels. Zt. s. f. Nervenheil Kunde*, 1925, **86–87**, 117.)

Reduced activity of the eosinophile cells is associated with the converse condition of "*dwarfism*" (Figs. 631, 632), though the condition is usually complicated by the results of deficiency in other pituitary hormones besides the growth factor. In the form known as the *Lorain-Levy* type there is a state of sexual infantilism; the dwarfs are of childish stature and proportions, though of normal intelligence; in girls, menstruation is delayed or absent. Dwarfism of the Lorain-Levy type is said to be associated with chromophobe adenomata, the depression of the hormone production being then ascribed to the pressure which results.

In the *Fröhlich-syndrome* (Fig. 633) there is adiposity and great apathy in addition to infantilism, and sexual development is conspicuously delayed in males. When the condition arises in adult life, the conspicuous features are fatness and loss of sex functions. It is usually due to a congenital tumour of the hypophysial duct and is of complex symptomatology.

Dwarfism may also be produced experimentally in animals by removal of the anterior lobe of the pituitary body when young. These hypophysectomised animals, of course, also show results of deprivation of other pituitary hormones, just as do some of the human cases of pituitary hypofunction. Thus some are sexually infantile (lack of gonadotrophic hormone), and these retain infantile appearance and proportions. Others, in which only growth is in abeyance, have normal adult proportions. The administration of growth hormone extracts to human dwarf children has resulted in resumption of growth.†

(2) **The Gonadotrophic Hormones.** It was discovered by Smith and Engle, and by Zondek and Aschheim in 1927, that the subcutaneous implanta-

* H. M EVANS and SIMPSON. *Amer. J. Physiol.*, 1931, **98**, 511, BÜLBRING *Q J. Pharm Pharmacol.*, 1938, **11**, 26.

† TAYLOR. *Endocrinol.*, 1938, **22**, 707.

tion of fresh anterior pituitary gland into young animals led to precocious sexual development, as a result of which the gonads not only matured early, but also, in consequence of their maturation, produced other hormones having important effects on the secondary sex characters and on the sex cycle. These hormones will be referred to in more detail in Chapter LII.

(3) **Lactogenic Hormone.*** Various other factors, to be dealt with later (Ch. LIII), are responsible for the initial growth and development of the mammary glands during pregnancy, but these effects do not culminate in secretion of milk. Once the growth of the glands has reached a suitable stage, however, secretion can be initiated and maintained by a hormone formed in the anterior pituitary. The name "*prolactin*" has been given to this hormone, which can be separated from most of the other pituitary constituents, and shown to be independent of them. Separation of prolactin as a crystalline protein has been claimed.†

It is assayed by its effect in causing a growth in the crop-gland of pigeons. The international unit of prolactin has been defined as the specific activity contained in 0·1 mg. of a standard preparation.

(4) **Thyrotrophic Hormone.‡** Extirpation of the pituitary body leads to an involution of the thyroid, reduction of about 30 per cent. in the basal metabolic rate, and a reduced nitrogen excretion. The result is due to the removal of a hormone, the thyrotrophic hormone, which exerts a stimulating effect on the thyroid. Conversely, thyroidectomy results in enlargement of the pituitary body. It has been suggested that myxœdema and cretinism may be primarily due to pituitary hypofunction, the thyroid being affected secondarily. The hormone can be prepared reasonably free from active impurities,§ and when injected leads in a few days to an increase up to + 35 p.c. in basal metabolic rate, accompanied by evidence of increased formation of thyroxin and by thyroid hyperplasia‖ and exophthalmos. Assay of the hormone is made by estimating the effect of five daily doses on the weights of the thyroids of immature female guinea-pigs (200 g.); the weight increases from 30 mg. to anything up to 100 mg., according to the dose.¶ The maximal effect on metabolism is reached after one or two weeks of daily injections, and thereafter dies down, and returns to the normal, or even falls below it. The reduction appears to be due to the presence, in the serum of the injected animals, of an anti-body, due to the antigenic action of the protein of which the hormone consists, or with which it is associated.

The hormone produces no effects on thyroidectomised animals. Thyrotrophic hormone is excreted in the urine, and may be recognised there after thyroidectomy; this may be due to increased production or to failure of fixation by the thyroid. A *parathyrotrophic* hormone has also been suspected.

(5) **Adrenotrophic Hormones.**** After hypophysectomy there is considerable atrophy of the inner layers of the suprarenal cortex which can be reversed by injection of anterior pituitary extracts, and which has its counterpart in the clinic in the small suprarenals seen in pituitary dwarfism.

* RIDDLE. *J. Amer. Med. Assoc.*, 1935, **104**, 636.

† WHITE, CATCHPOLE and LONG. *Science*, 1937, **86**, 82

‡ COLLIP. *J. Amer. Med. Assoc.*, 1935, **104**, 916.

§ ANDERSON and COLLIP, *J. Physiol.*, 1934, **82**, 11; CREEP. *Amer. J. Physiol*, 1935, **110**, 692.

‖ M. KROGH, LINDBERG and OKKELS. *Act. Path. et. Microbiol. Scand.*, 1932, **9**, 1

¶ ROWLANDS and PARKES. *Bioch. J.*, 1934, **28**, 1829.

** COLLIP. *J. Amer. Med. Assoc.*, 1935, **104**, 920; COLLIP et al. *Lancet*, 1933 (ii.), 347.

The loss of muscle tone following hypophysectomy is probably due to adrenal cortical atrophy, since it is restored by administrations of cortex extracts. A reciprocal connection is also indicated by the fact that after epinephrectomy, and in Addison's disease, there is a reduction in the number of basophile cells in the anterior pituitary.

Hypertrophy of the suprarenal cortex can be obtained by the injection of appropriate anterior pituitary extracts, and cortical hyperplasia is not infrequently seen in acromegaly. Cushing showed that in certain cortical adrenal tumours, associated with painful adiposity and reproductive disturbances, with hirsutes in females, there is frequently a basophile adenoma of the pituitary * (Fig. 634).

Since it has been shown that the suprarenal cortex has important effects on the metabolism of carbohydrate, fat and protein, the question has been raised whether some of the effects previously attributed to special pituitary

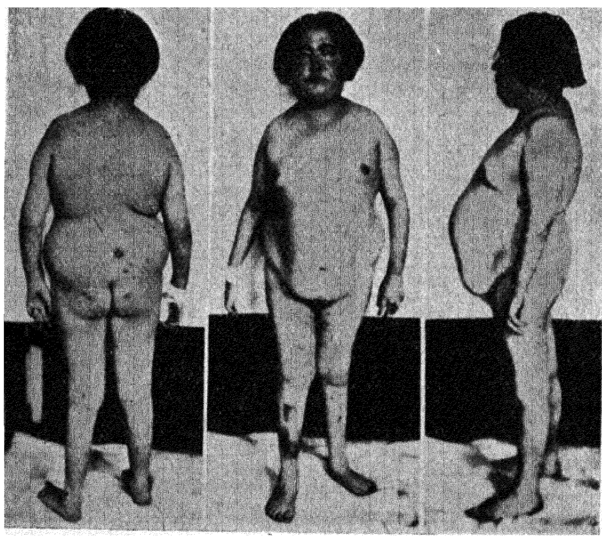

FIG. 634. Case of pituitary basophilism. Note the dark-coloured face and adiposity. (From CUSHING, *Bull. Johns Hopk. Hosp.*, 1931, **49–50**, 137.)

metabolic hormones (*see* below) may not be due to indirect effects of the adrenotrophic hormone

(6) **Metabolic Hormones.**† There appear to be at least four of these, three influencing the metabolism of carbohydrate and one that of fats.

CARBOHYDRATE METABOLISM is influenced by what has been called the *diabetogenic hormone* or "blood-sugar raising principle" (already mentioned on p. 1020). It has been observed that glycosuria, or even diabetes, may be associated with acromegaly, while in the Fröhlich syndrome there is increased carbohydrate tolerance ; it has been shown that hyperglycæmia and glycosuria may be produced after an interval of some days by the injection of extracts of the anterior lobe. When the pituitary is extirpated, on the other hand, the animals become very sensitive to insulin and even tend to develop hypoglycæmic symptoms if not liberally fed. The hormone has been said to be excreted in the urine, and to be particularly abundant

* CUSHING. *Lancet*, 1930, pp. 119, 175.
† COLLIP. *J. Amer. Med. Assoc.*, 1935, **104** 827, 916.

in the urine in diabetes, from which it can be removed by adsorption on kaolin. Since the extracts exert their action on thyroidectomised animals, the hormone is clearly distinct from the thyrotrophic one. Most of the extracts containing it, however, also contain the growth hormone, and *vice versâ*, though the two are believed to be distinct; the gonadotrophic hormone can be obtained free from it.

It seems certain that some cases of spontaneous hypoglycæmia are due to hypofunction of the anterior pituitary,* and on the other hand, it is possible that certain clinical cases of diabetes may be due to hypertrophy of the anterior lobe; the mode of action of the principle is unknown, though there are indications that it acts either through an effect on the central nervous system or direct on the liver. It is significant that the delay in its action is stated to be much shortened if it be injected directly into the cerebrospinal fluid. Others consider that it accelerates gluconeogenesis in the liver. Another apparently distinct hormone affecting the metabolism of carbohydrates is the *glycotrophic factor* (p. 1020), which reduces sugar tolerance, sensitises to adrenaline, and increases the resistance to insulin, but does not cause hyperglycæmia.

The third carbohydrate effect is the so-called "*pancreatrophic*" substance: it causes an increase in the insulin content of the pancreas.†

FAT METABOLISM. The presence of a hormone affecting fat metabolism was first indicated by the work of Burn and Ling,‡ who injected NaOH extracts of anterior pituitary into rats fed on butter. The injections caused considerable increase in the excretion of acetone bodies. It has since been shown that there is also an increase in the ketone bodies, particularly the β-hydroxybutyric acid, of the blood. The name "*ketogenic hormone*" has accordingly been given to the responsible principle. One of its effects seems to be an increase in glycogen storage in the liver. It acts on thyroidectomised animals and appears to be distinct from the other pituitary hormones, except possibly the glycotrophic one. It may be identical with a factor causing increased metabolic rate and fall of R. Q.§ It is probable that the close resemblance to clinical diabetes which results from dosage with the anterior pituitary is usually due to the combined actions of the carbohydrate and ketogenic hormones.

THE POSTERIOR LOBE

Extracts made from the pituitary, after removal of the anterior lobe, cause a rise of blood pressure when injected intravenously,|| and this observation formed the starting point for the investigation of the active principles of the posterior lobe, with its attached parts.¶

The physiological effects which can be produced by injection of pituitary 'posterior lobe' extracts ('pituitrin') containing all the hormones, and also histamine as an impurity, are as follows:

(1) Rise of arterial blood pressure (*pressor* action) with cardiac slowing, when large doses are injected intravenously into the anæsthetised animal. A second dose soon after the first produces a fall of pressure. The renal vessels are little if at all constricted, or may passively dilate. Most arterioles are constricted. Injected subcutaneously (0·5 c.c.) in man, there is no rise

* BEST. *Lancet*, 1934, 1155, 1216.
† MARKS and YOUNG. *Lancet*, 1940 (i), 493.
‡ BURN and LING. *J. Physiol.*, **65**, 191; 1930, **69** 19P.
§ O'DONOVAN and COLLIP. *Endocrin.*, 1938, **23**, 718.
|| SCHAFER and OLIVER. *J. Physiol.*, 1895, **18**, 230.
¶ GEILING, *J. Amer. Med. Assoc.*, 1935, **104**, 738.

of blood pressure, but intense pallor due to cutaneous vaso-constriction. The cardiac output may be diminished owing to constriction of the coronary vessels. According to Krogh, a certain content of pituitrin in the circulating blood is essential for the maintenance of the normal tone of the capillaries in the frog.

(2) Contraction of plain muscle generally. Administration of pituitary extract to a lactating animal leads to sudden expulsion of milk, due to contraction of the plain muscle in the walls of the milk sinuses. There is also contraction of the plain muscle of the alimentary canal. The production of contraction of plain muscle of the uterus is called the *oxytocic* action.

(3) Expansion of the melanophores (pigment cells) of the frog's skin (Hogben and Winton), so that the skin looks darker in colour. (Especially by extracts of the pars intermedia.)

(4) *Anti-diuretic Effect.* In anæsthetised animals, a brief diuresis follows the injection. This is due to the rise of blood pressure. If injected into a normal man who has received a large draught of water (1 l.) pituitrin delays for four or five hours the excretion of the water by the kidneys, so that it accumulates for a time in the tissues and may even cause an actual dilution of the blood. Ultimately a pathological condition, water poisoning, may develop owing to excessive retention of water. The effect appears to be due to an acceleration of water reabsorption in the kidney tubules. It also increases the excretion of certain ions, *e.g.*, Cl, as has been shown with the heart-lung-kidney preparation (p. 1064). There may be a temporary increase in the urine, with diminution of chlorides. The principle causing this water retention is much more abundant in mammalian than in other pituitaries.* The condition of diabetes insipidus, however brought about, may be relieved temporarily by the subcutaneous injection of a watery extract of the posterior lobe.

(5) Hyperglycæmia, glycosuria, lowered sugar tolerance, loss of liver glycogen, and an action antagonising that of insulin.

There is also a rise in the fat of the liver, and a decrease in basal metabolic rate. To what extent these effects are due to a specific hormone or to hormones from the anterior lobe is uncertain.

The best known actions are the pressor effect, the anti-diuretic effect, and the 'oxytocic' action on uterine muscle. For all these purposes pituitrin, in subcutaneous injection, is employed clinically.

It had long been disputed whether these actions are to be attributed to a single active principle, or whether more than one such hormone is concerned, as was claimed by Dudley. Kamm and others † settled the discussion in favour of the latter, and the separated oxytocic and pressor principles ("*pitocin*" and "*pitressin*") can now be obtained on the market. The cardiovascular and antiduretic effects, and the contraction of most of the plain muscle of the body are due to the pitressin fraction. The contraction of the uterus is a specific effect of the oxytocin, but the human uterus is not always equally sensitive to its action; in the early stages of pregnancy it is ineffective, but as pregnancy advances the sensitiveness increases and reaches a maximum at parturition; after delivery the uterus is again insensitive for some time.

The melanophore expanding principle is a separate substance, derived from the pars intermedia.

The unit of pressor activity is that of 0·1 c.c. of pituitary (posterior

* Heller. *J. Physiol.*, 1941, **99**, 246.

† Kamm *et al.* *J. Amer. Chem. Soc.*, 1928, **50**, 573.

lobe B.P.) extract. Milliunits are commonly employed in standardising, the tests being made on spinal cats.

The international unit of oxytocin is the activity of 0·5 mg. of standard dried posterior lobe, as tested on an excised guinea-pig's uterus under standard conditions.* The oxytocic principle has been concentrated by adsorption methods † and an activity of 225 units per milligramme reached; this gave a powerful contraction of the uterus in a concentration of 1 in 550 millions.

Functions of the Posterior Lobe. There now seems to be little doubt that the posterior lobe has as its main function the control of the water balance of the body, by regulation of the secretion of urine. As a subsidiary function it is regarded also as playing some part in the conduct of parturition. The pressor action is probably incidental, and is only seen when relatively large amounts of the pitressin fraction are injected.

DIABETES INSIPIDUS. In some disorders of the pituitary body or hypothalamus, diabetes insipidus occurs. This is a state in which large volumes, 10 to 30 l. daily, of dilute urine are passed: the urine shows no other

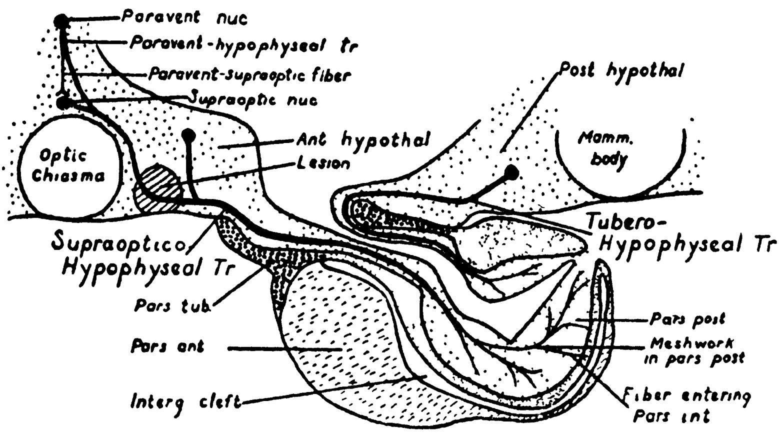

FIG. 635. Diagram of Medial Saggital Section of Hypothalamic Region of Cat showing Nervous Connections of Posterior Lobe of Pituitary Body. Bilateral Damage to the shaded area marked "lesion" causes Diabetes Insipidus. (RANSON, Harvey Lecture, 1936–37.)

abnormality than an excess of water; the large water output causes intense thirst and necessitates the drinking of large volumes of water. In experimental animals, diabetes insipidus can be produced in one of two ways, viz. by extirpation of the pars nervosa, or by bilateral section of the supraoptico-hypophyseal tract (Fig. 635).

When the tract is divided, polyuria occurs at once, lasts for about five days, and returns to stay permanently from about the twelfth day. It is then found that the pars nervosa has atrophied, and contains little or no active diuretic principle. This suggests that the posterior lobe is dependent for its function, and indeed for its existence, on the innervation which it receives by this tract.

When the posterior lobe, *i.e.* the pars nervosa, together with the median eminence and infundibular stem is extirpated, diabetes insipidus also follows, provided that no other damage is done, and that the extirpation is complete.

* BURN. Methods of Biological Assay, 1928. (Oxford: Med. Publ.)
† GULLAND and NEWTON. *Bioch. J.*, 1932, **26**, 337.

But *complete hypophysectomy does not produce this result*, though if an animal is completely hypophysectomised and then has anterior lobe tissue grafted under the skin, the polyuria occurs and lasts as long as the graft survives. It is therefore evident that in order to produce the state of diabetes insipidus the presence of the anterior lobe is as important as the total absence of functional pars nervosa, and it is immaterial whether the latter is made ineffective by actual removal, or by withdrawal of its nerve supply followed by atrophy. The condition is temporarily cured by the injection of small doses of pitressin.

The suppression of the diuresis by removal of the anterior lobe has its counterpart in the fact that the injection of anterior lobe extracts into normal animals often causes polyuria. This is possibly due to the general effects on metabolism, and on other endocrine organs, among which the suprarenal and thyroid must be counted. It has been shown that the cortical hormones of the suprarenal have an action opposing that of pitressin,* since they cause polyuria and increase chloride excretion, while adrenalectomy together with hypophysectomy leads to no polyuria

Thyroid extracts also exert a diuretic action, and after thyroidectomy the urine formation is reduced.

WATER REGULATION. When a large volume of water is drunk there is a lag in time between the occurrence of dilution of the blood and the onset of diuresis, and it has been supposed by Verney that this is due to the time taken for the anti-diuretic principle, present to begin with in the blood, to be appropriately diminished. This explanation does not suffice, however, because the same lag occurs in water diuresis in hypophysectomised dogs. But there is evidence that when the water of the body is reduced, there is a secretion of anti-diuretic substance into the blood, because such substances can be recovered from the urine of thirsting animals, except when they have been previously hypophysectomised,† or when their supraoptico-hypophyseal tracts have been severed.‡

As to the means by which the hormones of the pituitary posterior lobe are set free, there are results which suggest that it may be reflex. When the central end of the divided vagus nerve was stimulated, in the isolated perfused dog's head, an anti-diuretic principle could be demonstrated in the venous blood: pressor and oxytocic substance were also set free into the blood by afferent vagus stimulation. None of these substances was found in the blood from a hypophysectomised head under similar conditions.§

The afferent arc of this reflex was traced to the supraoptic region, and the efferent arc is, no doubt the supraopticohypophyseal tract.||

As a result of afferent vagal stimulation the pituicytes are found to undergo histological changes, such as a loss of granules, so that these cells may probably be regarded as the ones which secrete the hormones of the posterior lobe.¶

FUNCTION IN PARTURITION. As will be shown in a later chapter, the oxytocin of the posterior lobe may play a part in determining the onset of parturition. This is thought to be due to the fact that the uterus towards the end of pregnancy becomes increasingly sensitive to the contracting effect of oxytocin, and also possibly to the fact that at the onset of labour, there is an increased liberation of oxytocin into the blood stream. There is good evidence that after hypophysectomy delivery may be difficult.

* COREY, SILVETTE and BRITTON *Amer J Physiol*, 1939, **125**, 644.
† GILMAN and GOODMAN. *J. Physiol*, 1937, **90**, 113.
‡ INGRAM *et al* *Amer. J. Physiol.*, 1939, **127**, 544
§ CHANG *et al.* *Chinese J Physiol.*, 1937, **12**, 1; 1939, **14**, 161; 1938, **13**, 269.
|| HUANG *Chinese J Physiol.*, 1938, **13**, 367.
¶ WANG. *Chinese J Physiol*, 1938, **13**, 405.

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THE THYMUS

The Thymus forms two large masses in the anterior mediastinum, which in man grow rapidly up to the second year of life and then less rapidly ; involution begins at about the fifteenth year and steadily progresses, so that in later adult life only traces remain. As the gland atrophies the cortex disappears first and is replaced by connective tissue. The involution is delayed by castration and hastened by sexual activity, malnutrition and pregnancy. In certain cases of arrested development, or of general weakness in young people, the thymus has been found to be persistent. It contains a large amount of lymphatic tissue, and is therefore often associated with the lymphatic glands as the seat of formation of lymph corpuscles. The epithelial structures called Hassal's corpuscles, found in the medullary part of its lobules, have not had any function assigned to them Removal of the thymus appears to have no constant effect. The injection of extracts, administration by the mouth, or grafting of thymus tissue have in most instances given no regular and outstanding results An exception to this would appear to be provided by the work of Rowntree Clark and Hanson * They injected into rats an extract made with hot 0·5 p.c HCl from calf thymus. The injections were given daily and were continued in the progeny through several generations. The result was a definite increase in the rate of reproduction, and a striking acceleration in growth and in the attainment of maturity in the offspring. This was particularly striking by the fifth generation (see Table). When the administration was stopped the effect disappeared after one generation. It would appear that the effect of the extract is to excite all processes of maturation, especially in the offspring. Whether the extract

COMPARISON OF THYMUS-TREATED RATS WITH CONTROLS. (ROWNTREE, 1935.)

	Average birth weight, gm	Ears open, days	Teeth erupted, days	Hair appeared, days	Eyes opened, days	Testes descended, days	Vagina opened, days
Controls	4 6	2 5–4	9–10	14–17	14–17	35–40	60–70
F_1 . .	5 0	2	4–7	10–14	12–15	15–29	35–45
F_2 . .	5·2	1 2	1–2	4–6	4–6	5–9	23–31
F_3 . .	5 7	1	1	4–5	4–5	4–6	21–25
F_4 . .	5·7	1	1	3	3	4–5	18–19
F_5 . .	5·74	½–1	½–1	2	2	4–5	18
F_6 . .	4 7	At birth*	At birth*	1–2	1·5–2	3–4	16

* On first inspection or within twenty-four hours.

* ROWNTREE, CLARK and HANSON. *J. Amer. Med. Assoc.*, 1934, **103,** 1425.

would be effective if given by mouth is doubtful. In curious contradiction to these experiments, it has been stated that when tadpoles are fed on fresh thymus, their metamorphosis is retarded, though growth continues, so that giant tadpoles result. It is still doubtful whether the thymus should be counted as one of the endocrine organs.

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OTHER ENDOCRINE ORGANS

The internal secretions of the *pancreas* and of the *gonads* are dealt with elsewhere (Chapters XLVI and LII).

The *Pineal Gland* has so far not been proved to have any endocrine function.

CORRELATION OF ENDOCRINE ACTIONS

The functions of the individual organs of the body are correlated in two ways—by the action of the nervous system and by the action of hormones, and in the conservation of the constancy of the internal environment (homoiostasis) in its many different aspects, sometimes one and sometimes both means of correlation are brought into play. For example, the sugar level of the blood is kept approximately constant as a result of the action of hormones, such as insulin, adrenaline, the hormones of the suprarenal cortex, anterior pituitary and thyroid, and of the central nervous system acting through its effects on the liver, suprarenal medulla and pancreatic islets. It seems almost self-evident, in such a case, that each of these factors must not only be able to influence the blood sugar level, but must also in its turn be influenced by it, and/or by all the other factors that play a part: if this were not so, we should have not correlation, but metabolic chaos.

The rate at which insulin is secreted by the pancreas is not affected by altering the total amount of pancreas within reasonable limits, *e.g.*, by partial excision or by the in-grafting of as many as three extra glands, but only by the amount of sugar in the blood which reaches it.

The most remarkable examples of the inter-connections between endocrine organs are found in those instances in which the anterior pituitary plays a part, and of which we have already met with some examples. For instance, the anterior pituitary produces thyrotrophic hormone, which controls the growth and activity of the thyroid, while the thyroid hormone also exercises an influence on the size and activity of the anterior pituitary. But the interaction does not stop at a mutual one between two endocrine functions, but may echo widely throughout the whole endocrine system. Thus, thyroidectomy leads to enlargement, with hypofunction, of the anterior pituitary: this results in deficient formation of gonadotrophic hormones and so to depression of sexual functions, atrophy of sex organs, loss of secondary sex characters, and so on.

A brief calculation will show that the permutations and combinations of actions between the different hormones must have almost limitless possibilities, and it has in fact, as well as in fancy, been possible to postulate more than one endocrine "House that Jack Built."

Various types of functional association between the endocrine organs have been found, *e.g.* :—

(1) An organ, *e.g.*, the anterior pituitary, may produce a hormone which has the effect of maintaining or stimulating another endocrine organ, *e.g.*, the gonads.

(2) The hormone of one organ, *e.g.*, the gonads, may moderate and reduce the activity of another, *e.g.*, the gonadotrophic function of the anterior pituitary.

(3) Some hormones sensitise tissues to the action of other hormones *e.g.*, thyroxine sensitises to the action of adrenaline.

(4) Two hormones, *e.g.*, those of the ovary and that of the corpora lutea, may sometimes work to reinforce each other, and in other ways may be antagonistic.

In order to establish an effect of one endocrine organ, A, on another one, B, it should be shown that (1), extirpation of B produces at least as great a hypofunction as extirpation of A, and (2) the effects of extirpation of B cannot be counteracted by implantation or injection of A.

It should be noted that although hyperfunction is seen under pathological conditions in which adenomata of the endocrine glands are formed, it is not possible to produce such results of hormone excess by the grafting of additional endocrine tissue into a normal animal. This is because a hormone usually exerts a controlling action on the growth or activity of the gland which produces it. Thus, by giving large doses of thyroid, a state of hypofunction of the thyroid, with eventual atrophy of the gland may be produced. It is for similar reasons that a graft of an endocrine organ does not take well if by doing so the animal would then be provided with an excess of it, but takes best when the organ in question is deficient in amount or activity, or has been previously excised. In other words, an endocrine organ will not grow where it is not needed. When a graft is made into a normal animal it does not lead, even immediately, to liberation of excess of the hormone ; thus if the thyroid and parathyroids are grafted in by blood-vessel suture into a parathyroidectomised dog, the blood calcium rises to normal in a few hours, and stays there ; but if the graft is made into a normal dog there is no alteration in the blood calcium. This means that, as in other known instances, it is the level of a particular constituent in the blood which regulates the action of the endocrine organs in releasing their secretions.

Among the more important of the functional associations between endocrine organs the following may be mentioned :—

Anterior Pituitary and Gonads. The anterior pituitary, by its gonadotrophic hormone, maintains the structure and functions of the gonads, as will be explained in a later chapter, and as a result of the normal functioning of the gonads the sexual organs and secondary sex characters are also maintained in normal condition. Reciprocally, the gonads by their hormones, restrain the activity of the gonadotrophic functions of the pituitary.

Caution is needed, however, in the interpretation of some of these results. What has actually been shown is that, say, after administration of the gonad hormones, such as the œstrogens, the substance of the anterior pituitary will be found, when implanted into an immature animal, to be less potent than it usually is in provoking precocious sexual activity. This has, as stated, been interpreted as indicating that when the gonads are actively producing their hormones, these react on the anterior pituitary in such a way as to check the production of more of the gonadotrophic hormone there. But histological studies of the pituitary suggest that it is, in fact, secreting *more* actively

and that the absence of the gonadotrophic hormone may be due, not to slackening of secretion, but to exhaustion of the secretory reserves.

A similar criticism applies to many of the statements regarding inter-relations between endocrine organs.

ANTERIOR PITUITARY AND THYROID. By its thyrotrophic hormone the anterior pituitary controls the growth and activity of the thyroid, and the thyroid, reciprocally, restrains the rate of formation of the pituitary thyrotrophic hormone. After thyroidectomy the anterior pituitary hypertrophies, the basophiles increasing, and the gonadotrophic, thyrotrophic and lactogenic hormones diminishing.

ANTERIOR PITUITARY AND SUPRARENAL. The effect of the adrenotrophic pituitary hormone has already been discussed. A reverse action of the suprarenal on the pituitary is doubtful, though in Addison's disease there is a diminution in the basophiles.

ANTERIOR PITUITARY AND PARATHYROID. There is some doubt of the existence of relationship between these two glands.

ANTERIOR PITUITARY AND PANCREATIC ISLETS. This pancreotrophic hormone has already been mentioned (p. 1131).

THYROID AND GONADS. Although there is no doubt that alterations in thyroid activity affect the gonads and all that depends on them, it is not quite clear to what extent the relationship is an indirect one in which the anterior pituitary is concerned. Generally speaking, thyroidectomy causes hypofunction of the gonads (but also of the anterior pituitary), a condition which is rectified by the administration of thyroid. In normal animals small doses of thyroid stimulate the gonads to increased functional activity, but large doses depress sex functions. Castration causes in some species a state of hypofunction of the thyroid, while the ovarian follicular hormone may cause hyperthyroid activity.

SUPRARENAL AND GONADS. The important effects of the suprarenal on the sex functions has already been mentioned, and is not surprising in view of the fact that cortical hormones include some of the sex hormones, and in all cases are chemically closely related to them. Tumours of the cortex, with overaction of the cortical layers, produce the remarkable cases of virilism with hirsutism in women; surgical removal causes a complete cure. In some cases the anterior pituitary is perhaps the prime offender, as in pituitary basophilism, in which, as a secondary effect, the suprarenal cortex is involved.

Nervous Control of Endocrine Organs. Although the endocrine organs exert their effects on other organs, including other endocrine organs, through the blood stream, they are not free from control by the central nervous system. The ideas of hormonal and nervous control are, however, closer to one another now than in the past, for, as we have seen, it is probable that, in the last resort, the transference of nervous action is probably effected by the agency of chemical transmitters, such as acetyl choline and adrenaline, and this may be viewed as a kind of local endocrine activity.

The suprarenal medulla is certainly under the control of the nervous system, and is reflexly excited to activity by a variety of afferent stimuli, the efferent path being by way of twigs from the splanchnic nerves. Other instances are less definite, but in some respects the anterior pituitary provides an example. In some mammals, such as the rabbit, the maturation and discharge of ova does not occur until about twelve hours after copulation has taken place. It does not occur even then if the pituitary is extirpated within half an hour of coitus,* but can be produced at any time by the

* FEE and PARKES. *J. Physiol.*, 1929, **67**, 383

injection of an extract of the anterior pituitary, or by electrical stimulation of the brain.*

The reflex discharge of antidiuretic and oxytocic substances into the blood by vagus stimulation, already mentioned, is similar proof that the posterior pituitary is also under the control of the nervous system.

The islet tissue of the pancreas is another instance of nervous control, for although the secretion of insulin is mainly controlled by the blood sugar level, acting directly on the islets, there is probably a second line of action, perhaps reserved for emergencies, and in which the vagi act as secretory nerves to the islet tissue.

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B. REPRODUCTION

CHAPTER LI

THE FORMATION OF GERM CELLS

In multicellular organisms, the cells which conjugate to form a new cell, capable of developing into an individual, are of two kinds. One, which has generally a certain amount of reserve material stored up in its cytoplasm, is the female element and is called the *ovum*. The other cell, which consists of little more than nuclear material, is the male element and is called the *spermatozoon*. Both kinds of cells are derived from the germinal epithelium which can sometimes be traced directly back to the first divisions of the fertilised egg. The use of the reserve material in the ovum is to serve as food for the developing individual The ripe ovum and spermatozoon cannot be regarded as complete cells, but rather as half-cells. Before their union, or conjugation, both male and female germ cells undergo certain important changes which further differentiate them from the somatic cells of the individual. The essential differences between a ripe germ cell and a somatic cell can be best seen by a study of the nuclear changes which precede their formation.

Mitotic Division. In division, the nuclei of all somatic cells, undergo a series of changes which are similar throughout both animal and vegetable kingdoms (Fig. 636), and result in the production of qualitatively identical daughter nuclei.

The nucleus of the resting cell in its vegetative condition is generally separated from the cytoplasm by a nuclear membrane, and contains irregular masses of chromatin. In the cytoplasm of most animal cells may be seen a small particle known as the centrosome. When division is about to take place, the clumps of chromatin arrange themselves into a filament which forms a continuous skein, the 'spireme stage.' This then breaks up into a number of segments, often V-shaped, the chromatin filaments or *chromosomes*. Each of the filaments, in large nuclei, may often be seen to be composed of rows of granules. While this change has been occurring, the nuclear membrane in most cases disappears, and the centrosome outside the nucleus divides into two parts which travel to opposite ends of the nucleus. Round each centrosome, the cytoplasm is modified and presents a radiate appearance, the *aster*, while joining the two centrosomes is a spindle of fine fibres, the *achromatic spindle*. The V-shaped segments of chromatin arrange themselves in a circle at the equator of the spindle midway between the two centrosomes. Each of the loops then *splits longitudinally*, and each half travels towards one or other of the centrosomes, thus forming two daughter nuclei. The half-loops then join to form a skein, and may return to the condition of a resting nucleus. The entire process, as seen in tissue cultures, takes about two or three hours, though probably about twelve hours elapse between the commencement of division in a cell and the beginning of renewed division in the daughter cells. These different phases in division are presented by all somatic cells, and have received the following names:

(1) *Prophase* (the formation of the spireme and of the achromatic spindle, and the breaking up of the spireme into chromatin loops or chromosomes). This phase occupies from ten to thirty minutes.

(2) *Metaphase* (the splitting of the chromosomes). This takes from two to twelve minutes.

By the action of the alkaloid colchicine the division of cells is arrested at the

metaphase. This is an advantage when we wish to confirm the occurrence or increase of cell division, because after a few hours a decisive number of cells in metaphase will have accumulated.

(3) *Anaphase* (the travelling of each half-chromosome to the extremity of the spindle), which occupies about three to five minutes.

(4) *Telophase* (the retrogressive changes, leading to the conversion of the chromatin

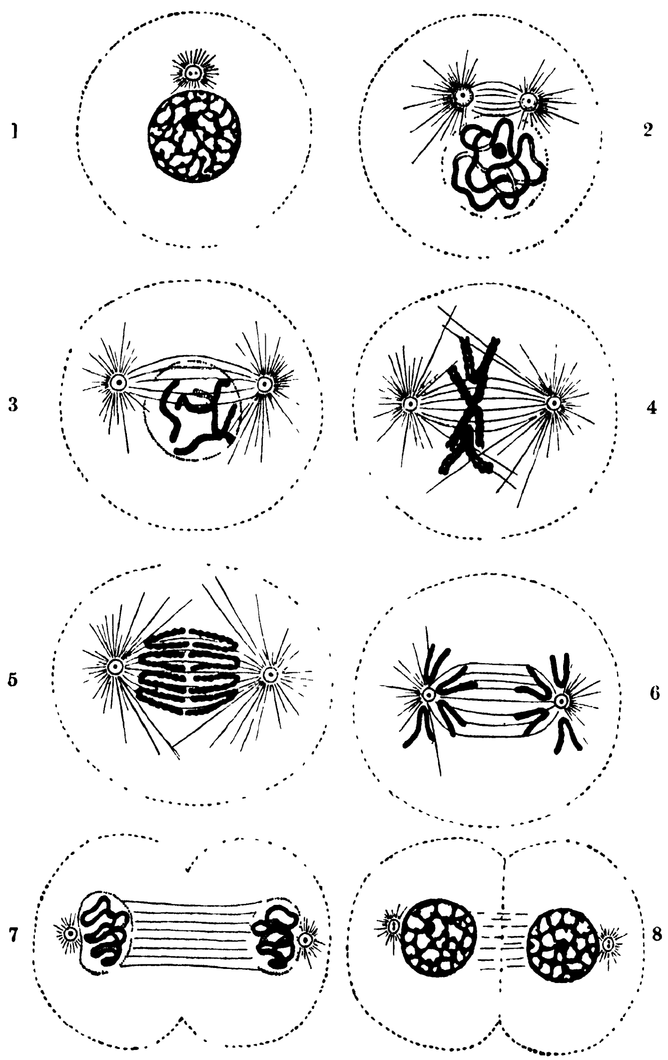


FIG. 636. Diagram showing the Changes which occur in the Centrosomes and Nucleus of a Cell in the Process of Mitotic Division. (SCHAFER.) The nucleus is supposed to have four chromosomes.

filaments into an ordinary resting nucleus, which are accompanied or preceded by a division of the cytoplasm across the equatorial part of the spindle). The latter part of the process takes about three to twelve minutes, but the retrogressive changes take from thirty minutes to two hours.

When the spireme has broken up into separate chromosomes, it is possible to count them, and it is found that the number present in any cell (*diploid number*), is constant for the species. The chromosomes are not all alike in appearance or in potentialities, but are present in pairs that are alike. Thus, every human somatic cell has 48 chromo-

somes, *i.e.* 24 pairs of chromosomes in its nucleus.* Identical numbers are found in types so far apart as the ox, the guinea-pig, and the onion. In the mouse, the salamander, and the lily the number is twenty-four. Other types, such as the crustacean Artemia, are said to have as many as 168 chromosomes, the much-investigated fruit-fly, *Drosophila melanogaster,* has eight, while in Ascaris the cells contain only two to four chromosomes. There is usually an even number of chromosomes, the chromosomes of each pair being similar in size and shape. On division, each chromosome gives rise to daughter-chromosomes of exactly similar shape and size. The somatic type of cell division, called *mitosis,* seems to be adapted to ensuring an equal qualitative as well as quantitative distribution of the nuclear chromatin among the daughter cells resulting from the division of any cell. As we have seen in an earlier chapter, the nucleus, by its interaction with the cytoplasm, determines the processes of assimilation and growth of the whole cell. For the preservation of type in cell division it is therefore essential that the nuclei of the daughter cells shall be identical in all respects with the nucleus of the mother cell.

Reduction Division. Sexual reproduction involves the conjugation of two cells, with union of their nuclei. If each of these nuclei consisted of the

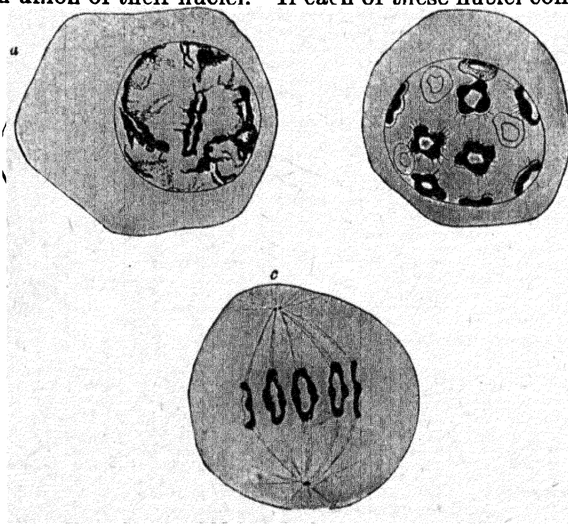


Fig. 637. Three Stages of Meiosis in Spermatocyte of Triton. (Moore.)
a. Germinal condition of chromosomes.
b. Gemini arranged in quadrate loops or tetrads.
c. Separation of tetrads into the duplex chromosomes of the daughter nuclei.

normal number of chromosomes, the fertilised egg cell would contain double the number characteristic of the species, and since these chromosomes would divide by splitting, the number of chromosomes in each cell would be doubled with each generation. This doubling is obviated by the fact that, in the formation of the germ cells, the ovum and spermatozoon nuclei undergo a special type of division, which leads to the reduction of the chromosomes in the sexually mature cell to one half of the number characteristic of the species. This mode of cell division is often called 'reduction division,' or 'meiosis' (Figs. 637 and 638).

We may take as an example the development of spermatozoa. The mother cells of the spermatozoa, the *primary spermatocytes,* divide twice, giving rise to four daughter cells, the *spermatids,* each of which develops into a functional spermatozoon. In the nuclear changes preparatory to the first division, the pairs of similar chromosomes become fused together (synapsis). At the first, or reduction division, the members of each pair, instead of being split longitudinally, are merely separated again, so that each

* Evans and Swezy. *Mem. Univ. Calif.*, 1929, 9, No. 1, p. 65.

of the resulting cells receives one member of each pair and hence contains what is called the *haploid* number which is only half the somatic (or diploid) number of chromosomes. At the second division, these are split in the usual way, so that the ripe germ cells also contain the haploid number of chromosomes, *e.g.* 24 in man. The distribution of the paired chromosomes between the daughter cells at reduction provides the possibility of qualitative differences between them, since the chromosome which goes to each daughter cell represents one of the two which form a pair in cells of the somatic type. If we indicate four normal chromosomes as *a*, *b*, *c*, *d*, of which *a* and *b* form one pair and *c* and *d* form the other, in ordinary somatic division each daughter cell would also contain chromosomes which may be represented as *a*1, *b*1, *c*1, *d*1, and *a*2, *b*2, *c*2, *d*2. But on reduction one daughter cell receives *a* and *c*, while the other daughter cell receives *b* and *d*

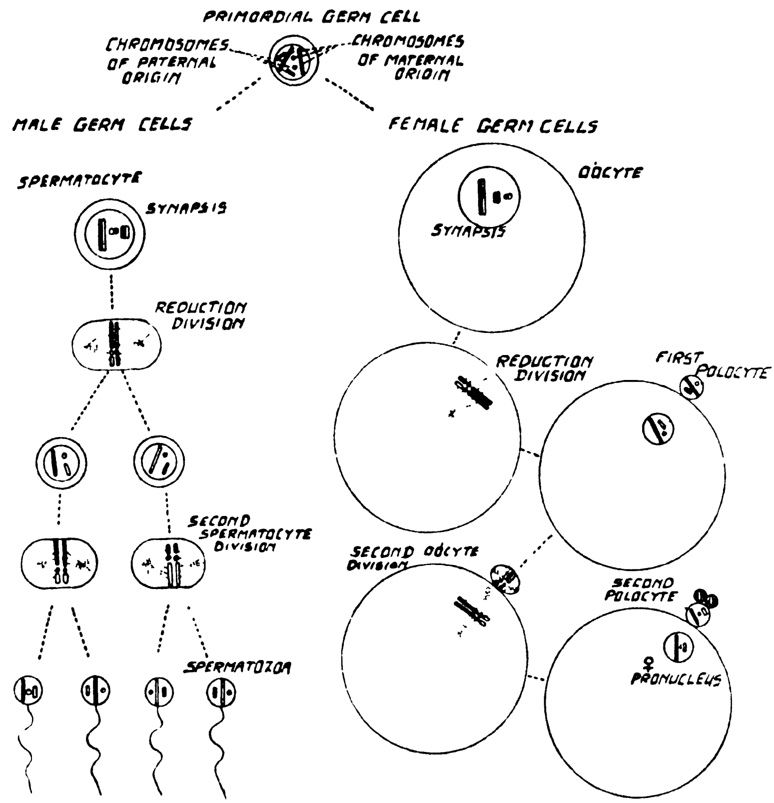


FIG. 638. Diagram to illustrate Formation of Germ Cells with Haploid Number of Chromosomes (three). (LUDFORD.)

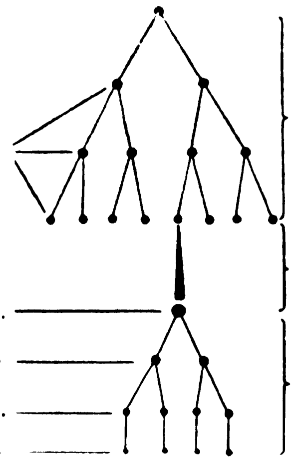
in the usual way. Exactly similar changes take place when there are larger numbers of chromosomes, one member of each pair of somatic chromosomes finding its way into the ripe spermatozoon (Figs. 638 and 639).

The mitochondria and Golgi apparatus can be traced throughout all the stages of spermatogenesis, and are approximately shared out in the cell divisions. In the formation of the spermatozoa from the spermatids, well-marked changes occur in the cytoplasmic inclusions. The tail of the spermatozoon arises in association with one of the centrioles. In the mature spermatozoon the head is derived from the nucleus of the spermatid; the acrosome, which forms a cup of variable shape according to species, is formed in association with the Golgi apparatus, in a manner somewhat similar to that of the formation of granules in gland cells. The middle piece is of cytoplasmic origin and composed largely of mitochondria in spiral formation.

In the ovum, during maturation, analogous changes take place. Two successive

cell divisions occur as in the formation of spermatozoa, but the daughter cells are of very unequal size. In the first or reduction division, the chromosome pairs separate as in the spermatocytes, so that each of the daughter cells contains the haploid number of chromosomes, and, as in the male, each of them represents one of a pair of similar chromosomes present in the somatic cells. The larger of the two resulting cells, which retains most of

Primordial germ cell.



Spermatogonia

Division period (the number of divisions is much greater).

Growth period

Primary spermatocyte.

Secondary spermatocyte

Maturation period.

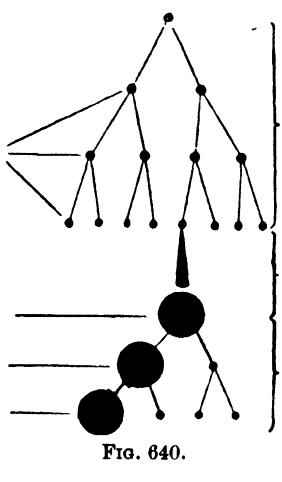
Spermatids.

Spermatozoa

FIG. 639.

the cytoplasm, is still called the ovum, while the smaller one is spoken of as the 'first polar body' or 'first polocyte.' The ovum now divides again and throws off a second polar body, the division being of the usual homotypical variety. The first polar body also divides, so that, from the original ovum, four cells are produced, one of which retains the greater part of the cytoplasm, while the others are extruded, and degenerate

Primordial germ cell.



Oögonia

Division period (the number of divisions is much greater).

Growth period.

Primary oöcyte or ovarian egg.

Secondary oöcytes (egg and first polar body).

Maturation period.

Mature egg and three polar bodies.

FIG. 640.

(Figs. 638 and 640). The only difference, therefore, between the formation of ovum and spermatozoon is that in the former case three of the cells formed by the division of the primitive ovum are abortive, whereas in the spermatozoon all four daughter cells produced from the spermatocyte remain functional. Since the nuclei of the mature ovum and spermatozoon contain only the haploid number of chromosomes, they are sometimes spoken of as *pro-nuclei.*

In oögenesis, the mitochondria increase in numbers and are evenly distributed in the cytoplasm of the ripe cell. The Golgi apparatus similarly becomes spread out, and in some cases yolk is formed in association with it; in the mammal, however, it merely spreads out, usually near the periphery.

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FERTILISATION

The essential features of fertilisation, *i.e.* the union of the sexual cells, are best studied in some of the lower invertebrates, such as ascaris or echinoderms. In the latter, fertilisation takes place in the sea-water, into which both ova and spermatozoa are extruded. The ovum of the echinoderm consists of a naked mass of protoplasm. Of the countless hordes of spermatozoa which may be in the neighbourhood of a given ovum, only one, as a rule, enters. As soon as the spermatozoon has entered the ovum, a tough membrane is rapidly formed round the latter, so preventing the entrance of any further spermatozoa. The head of the spermatozoon enters the egg, while the tail atrophies and disappears. The head of the spermatozoon enlarges and assumes the character of a nucleus, the dense mass of chromatin breaking up first into a thread and then into the characteristic haploid number of chromosomes (Fig. 641). The egg now contains two pro-nuclei, exactly similar in appearance, one derived from the male and the other belonging to the egg itself. The two pro-nuclei approach one another and join. In many cases, there is an apparent fusion of the substance of the two nuclei. In others, the chromatin filaments of male and female simply lie side by side, forming a complete nucleus with the diploid number of chromosomes. The fate of the remainder of the spermatozoon is uncertain: it has been suggested that the acrosome plays some essential physico-chemical part in fertilisation, but there are no indications of what this may be. In some rare cases, the mitochondria of the spermatozoon have been seen to enter the cytoplasm of the ovum and mingle with those already there. Fertilisation is rapidly followed by an enormous increase in metabolism, *e.g.* in the oxygen consumption, and by cell division. Each of the chromosomes splits longitudinally, half going to each of the daughter cells, and this process is repeated throughout the succeeding divisions which result in the formation of the new individual. Thus, every cell of the body contains a nucleus of which exactly one half is paternal and the other maternal in origin.

The strong impetus to cell division given by the process of fertilisation has naturally aroused much curiosity as to its intimate character. It might be thought that, for cell division to commence, a normal number of chromosomes is essential. As against this explanation may be adduced the fact that, in many animals, *parthenogenesis* occurs. The female pro-nucleus may, under certain conditions of environment or nutrition, start dividing and give rise to an embryo, each cell of which contains only the haploid number of chromosomes. In other cases of parthenogenesis, only one polar body is extruded, or the second polar body joins again with the female pro-nucleus. In these cases, the ovum contains a nucleus, with a diploid number of chromosomes, which divides and produces an individual resembling that resulting from the union of ovum and spermatozoon. It has been suggested that the impetus to division is given by the entry of the spermatozoon itself. In the series of divisions which precede the formation of the female pro-nucleus, the centrosome of the ovum generally disappears, whereas

in the formation of the spermatozoon, the centrosome persists and forms the middle part of the spermatozoon. In many cases, the centrosomes divide in the spermatozoon itself, so that this contains two centrosomes when it enters the egg. These two centrosomes then become the centres of attrac-

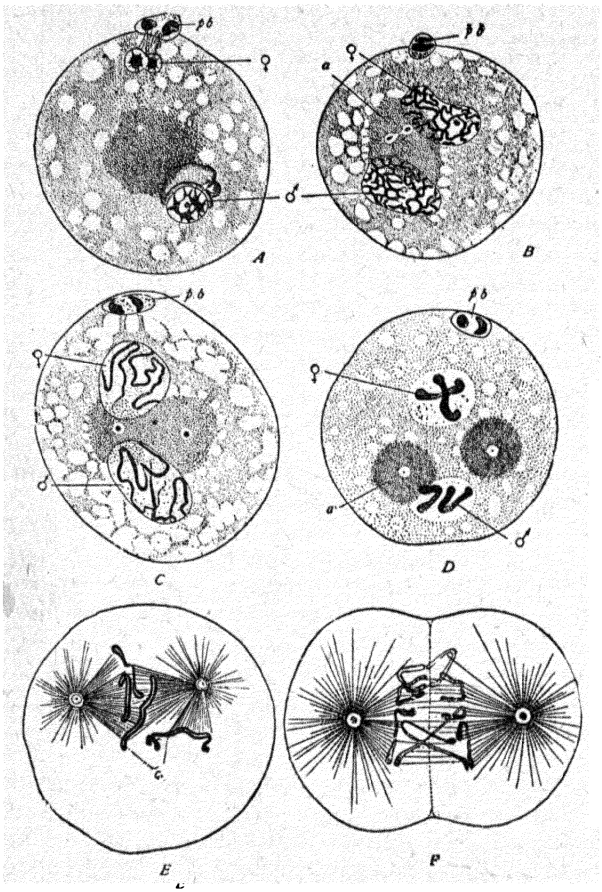

FIG. 641. Fertilisation and First Division of Ovum of Ascaris Megalocephala. (Slightly modified from BOVERI and WILSON.)

A. Second polar globule just formed: the head of the spermatozoon is becoming changed into a reticular nucleus (♂), which, however, shows distinctly two chromosomes; just above it, its archoplasm is shown: the egg nucleus (♀) also shows two chromosomes. B. Both pro-nuclei are now reticular and enlarged; a double centrosome (*a*) is visible in the archoplasm which lies between them. C. The chromatin in each nucleus is now converted into two filamentous chromosomes; the centrosomes are separating from one another. D. The chromosomes are more distinct and shortened; the nuclear membranes have disappeared; the attraction spheres are distinct. E. Mingling and splitting of the four chromosomes (*c*); the achromatic spindle is fully formed. F. Separation (towards the poles of the spindle) of the halves of the split chromosomes, and commencing division of the cytoplasm. Each of the daughter-cells now has four chromosomes; two of these have been derived from the ovum nucleus, two from the spermatozoon nucleus.

tion spheres. They diverge, and between them is formed an achromatic spindle, along the equator of which the chromatin filaments of male and female pro-nuclei arrange themselves. It is doubtful, however, how far the centrosome can be regarded as a permanent cell structure.

In echinoderm eggs, various modes of treatment will lead to the appear-

ance of attraction spheres in the cytoplasm, and even to division of the non-fertilised egg. Loeb suggested that the impetus by the spermatozoon is essentially due to a destructive action, which may be chemical in nature, on the cortical layer of the ovum. By the addition of mild cytolytic agents to the medium in which the eggs are contained, Loeb succeeded in imitating exactly the changes which normally need the entrance of a spermatozoon for their occurrence. On immersing the eggs in a weak solution of formic or lactic acid, a membrane is formed. The eggs are then taken from the acid, placed in concentrated sea-water for a short time, and then removed to ordinary sea-water. Division rapidly occurs, with the production of the normal larva. This artificial 'fertilisation' is called *artificial parthenogenesis*.* Loeb suggests that the spermatozoon brings with it enzymes or other chemical substances, which excite the egg nucleus and cytoplasm in the same way as the chemical measures adopted for this artificial induction of segmentation.

Mechanical damage to the outer part of the ovum may also lead to parthenogenetic development, as for instance of the unfertilised frog's egg when punctured with a needle.

* LOEB. *Amer. J. Physiol.*, 1900, **3**. 135 ; *Univ. Calif Publ.*, 1905, **2**, 83, 89, 113.

CHAPTER LII

REPRODUCTION IN MAN AND OTHER MAMMALS

HORMONIC INFLUENCES IN THE DEVELOPMENT AND FUNCTIONING OF THE REPRODUCTIVE ORGANS

EVEN within the mammalia the phenomena of reproduction show great variations from species to species. Some species breed only at certain seasons of the year, others at any season. The number of offspring produced also shows a species variation, and so do many details of the reproductive process. In the action of the hormones concerned, however, there is a good deal of common ground, and many of the observed differences between species may be traced to different quantitative or different time relations in the production of the essential hormones.

In the development and functioning of the reproductive apparatus the principal part is played by the hormones produced in the pituitary anterior lobe and in the reproductive organs themselves.

Puberty is the transition stage on the way to full sexual maturity. The onset of puberty is due to the action of the gonadotrophic hormones which now begin to be formed in the anterior lobe of the pituitary body, and which provoke the changes in the gonads which culminate in maturation of the germ cells, *i.e.* ripe spermatozoa and ova produced in the testis and ovary respectively. A further example of endocrine influence is found in the effects exerted by the hormones of the gonads themselves upon the other parts of the reproductive apparatus and upon the body generally. Thus, castration or spaying (oöphorectomy), *i.e.* removal of the testes or ovaries, if carried out before the time of puberty, prevents the development of the secondary sexual characters, which normally occurs at this epoch. In the human species, this period is marked, or preceded, in the male by increased growth of the skeleton, by growth of the larynx, leading to a lowering in pitch of the voice, by the growth of hair on the face and pubes, by development of the external genitalia, and by the development of sexual desire. In the female, at puberty we find enlargement of the breasts, attended by some growth of the mammary glands and by a moulding of the whole form, making it more fit for the bearing of children. The chief sign of puberty in the female consists in the periodic changes in the uterus, which in the human subject and the apes give rise to menstruation, *i.e.* a flow of blood and mucus from the genital organs, lasting three to five days and repeated about every four weeks. The various secondary sex characters are largely the consequences of the action on the body of divers hormones which now begin to be produced in the maturing gonads. Menstruation persists, except during pregnancy and early lactation, so long as the ovary is functional. The activity of the ovary comes to an end between the forty-fifth and fiftieth year ('the climacteric,' 'menopause,' or 'change of life'). With the cessation of its activity, menstruation also stops, and the uterus undergoes atrophy. Castration after puberty, though not causing any change in the skeleton, which has already assumed its permanent form, brings about retrogressive

changes in the other genital organs, analogous to those occurring in the female at the climacteric.

In animals, the series of changes known as the *œstrus cycle*, one phase of which includes the phenomena of 'coming on heat,' is in certain respects analogous with the menstrual cycle in the human female, and, like this, depends on the normal activity of the ovary. It is permanently abolished by extirpation of the ovaries, but may be reinduced by implantation in the peritoneum of an ovary from another animal of the same species or by injection of the appropriate ovarian hormone. This fact shows that the changes in the uterus responsible for 'heat,' as well as for menstruation, are independent of any nervous connections between the ovaries and the rest of the body, and must therefore be brought about by the circulation in the blood of hormones produced in the ovaries. According to some authors, the essential structures for the production of these genital hormones are the 'interstitial cells' found in both the testes and ovaries of various animals. These interstitial cells are not, however, universally present.

The chemical correlations between the ovaries and the other organs concerned in reproduction are perhaps best shown in the changes which attend *pregnancy*. This is accompanied by a great development, first of the mucous membrane, and later on of the muscular wall, of the uterus. The mucous membrane thickens, and so forms a bed for the developing fertilised ovum. With this growth of the uterus, there is a corresponding growth of the other parts of the genital tract, *e.g.* the vagina. At the same time, rapid changes take place in the mammary glands. These changes may be studied experimentally in the rabbit, in which gestation lasts only about thirty days. In a virgin rabbit of a year old, it is difficult, with the naked eye, to see any trace of the mammary gland, which consists entirely of ducts lined with a single layer of flattened epithelial cells. Four or five days after fertilisation, when it is still impossible with the naked eye to discover any embryos in the swollen uterine horns, on reflecting the skin from the abdomen each mammary gland appears as a circular pink area, about 3 cm. in diameter. On section, the gland consists of ducts which are in an active state of proliferation. By the ninth day, the whole abdomen is covered with a thin layer of glandular tissue; by the twenty-fifth day, this tissue is ½ cm. in thickness and consists for the greater part of secreting alveoli. At full term, the alveoli contain ready-formed milk.

This hypertrophy of the mammary glands during pregnancy occurs after complete division of all possible nervous paths between the glands and the ovaries or uterus. If two animals are united like Siamese twins (parabiosis), and so have blood in common, and one becomes pregnant, the mammary glands of both develop and form milk. Ancel and Bouin first brought forward evidence that the corpus luteum is intimately concerned with the growth of the mammary glands, and may indeed cause a certain degree of hypertrophy of these glands in the entire absence of any product of conception within the uterus. As first shown by Heape, discharge of an ovum and formation of a corpus luteum occur in the rabbit only as a result of copulation. The same effect may sometimes be produced by artificial rupture of a ripe follicle, whereupon there is a development of the mammary glands. If no impregnation has taken place (*e.g.* if the buck has been sterilised by ligature of the vas deferens), the glands develop for fourteen days and then begin to atrophy. This period corresponds to the period of active growth of the corpus luteum. The limited growth of the glands which occurs at puberty must be connected with the growth of ripe ova or with the growth of the corpus luteum.

There are also definite relationships between the activity of the sexual organs and that of certain other endocrine organs. Thus, castration at an early age leads to persistence of the thymus gland, which normally atrophies just before the sexual organs commence their functional activity. The existence of a connection between the thyroid and the ovaries has been a popular belief for 2000 years. In many individuals, the thyroid is perceptibly enlarged at each menstrual period. On the other hand, extirpation of the thyroid before puberty, brings about, among the other signs of cretinism, failure of development of the ovaries, so that puberty is partially or completely delayed. Hypertrophy of the pituitary body in pregnancy and in menstruation, and enlargement and increase of oxyphile cells in its anterior lobe after castration, as well as the inhibited development of the external genitalia in hypo-pituitarism, and many facts derived from laboratory experiments illustrate the fundamental relation between the pituitary body and the sex glands. There are also close relations with the suprarenal cortex, as shown, for example, in the sexual precocity associated with tumours of that tissue.

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THE FEMALE REPRODUCTIVE ORGANS

The essential organ of reproduction in the female is the ovary, the seat of production of the ova. The accessory organs in the mammal include the oviducts or Fallopian tubes, the uterus, in which the fertilised ovum is retained during the period of pregnancy, in which it develops, the vagina, which is adapted for the reception of the penis in the act of copulation, and the external genitalia.

Among the accessory organs we may also reckon the mammary glands, which undergo a special development during pregnancy, and serve for the nourishment of the young individual during the first period of extra-uterine life.

OVULATION. The discharge at certain periods, into the peritoneal cavity, of a ripe ovum, is called ovulation. It only occurs during the sex life. At the time of its occurrence an electrical potential difference is detectable between the cervix or vaginal epithelium and a point at the surface of the body,* but the significance of this is not known.

At birth the ovary consists of a stroma of spindle-shaped cells, and is covered by a layer of cubical epithelium (the germinal epithelium) continuous with the mesothelium lining the general peritoneal cavity. Embedded in the stroma, but especially numerous just underneath the epithelium, are a vast number of 'primordial follicles.' These are formed during fœtal life, by downgrowths of the germinal epithelium. They may perhaps also be formed continuously throughout the whole sexual life,† in a similar manner, though this is not the current belief. Of the cells produced in this way from the germinal epithelium, some undergo enlargement to form the primordial ova, while the others are arranged in single layers of flattened nucleated cells, the 'follicular epithelium,' as a sort of capsule to each ovum. Of the primordial follicles, about 70,000 are to be found in the ovary of the newborn child, and during the woman's life some 500 of these follow their full course and become ripe ova. The remainder are destroyed. During the first twelve to fourteen years of life, although some primordial follicles are already

* BURR *et al* *Yale J. Biol. Med.*, 1937, **10**, 155

† EVANS and SWEZY, *Mem. Univ. Calif.*, 1932, **9**, 119.

developing into well-formed Graafian follicles, with growth of the ova, these do not attain maturity, but retrogress and form 'atretic' follicles.

The maturation of the ova, with their containing Graafian follicles, first occurs with the onset of puberty. The first stage in the growth of the follicle is a proliferation of the follicular epithelium, the cells of which become cubical and are arranged in several layers round the ovum. At one point in the mass of cells surrounding the ovum, a cavity appears, filled with fluid, the *liquor folliculi*. The epithelium thus becomes separated into two parts, viz., the *membrana granulosa*, several layers thick, lining the whole follicle, and the

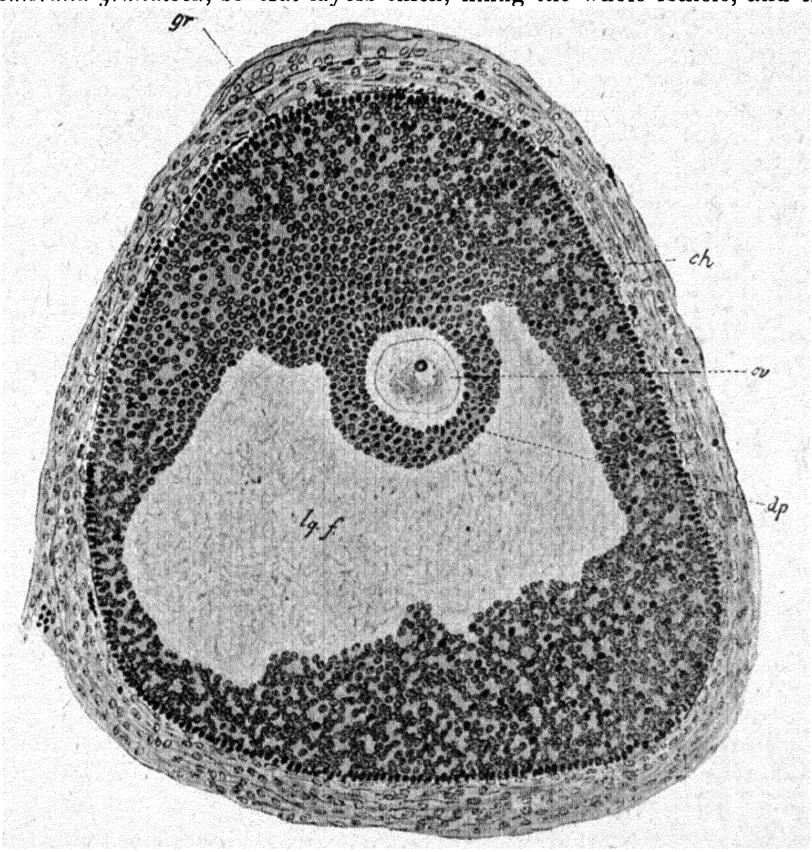

Fig. 642. Graafian Follicle of Mammalian Ovary. (Prenant and Bouin.)
ov. Ovum. *ch.* Theca.
dp. Discus proligerus. *gr.* Membrana granulosa.
lq.f. Liquor folliculi.

discus proligerus, a mass of cells attached to one side of the follicle, in which is embedded the ovum (Fig. 642). Round the growing follicle, the stroma assumes a concentric arrangement and forms a capsule, of which the internal layer consists chiefly of spindle-shaped cells richly supplied with blood vessels —the *theca interna*—while the outer layer—the *theca externa*—is made up of a tough fibrous tissue. With the growth of the follicle, the ovum also becomes larger and surrounds itself with a distinct membrane, known as the *zona pellucida*. This membrane presents a fine radial striation, which is supposed to indicate the existence of canals, through which the ovum may obtain sustenance from the surrounding cells of the follicular epithelium. The

nucleus also becomes larger, and forms the *germinal vesicle*, containing a well-marked nucleolus—*the germinal spot.* The mature Graafian follicle projects from the surface of the ovary as a transparent vesicle about the size of a pea. (Its diameter is about 15 mm. in the human.) In the process of growth, the ovum has increased from a diameter of 25μ to 200μ. Before the ovum can undergo fertilisation, the double division of the nucleus, or germinal vesicle, has to take place, which leads to the formation and extrusion of the two polar bodies. This process probably occurs just before or just after the discharge of the ovum from the ovary.

With increasing size of the Graafian follicle, the membrane covering it becomes progressively thinner and more vascular, and the ovum moves towards its outer surface. At certain periods, or under certain conditions, the membrane ruptures, and the liquor folliculi, with the ovum, is discharged into the peritoneal cavity, the ovum being, at first, still surrounded by an

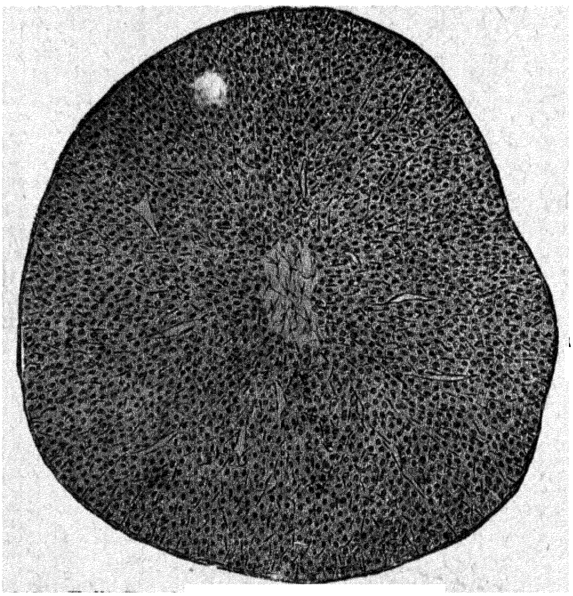

FIG. 643. Fully Developed Corpus Luteum of the Mouse. (SOBOTTA.)

adherent mass of the cells of the discus proligerus. In some animals, this process of *ovulation* occurs at definite periods of the year. In others, such as the ferret * and rabbit,† the occurrence of ovulation depends upon coitus taking place during the period of sexual activity. It is probable that, in the human female, ovulation occurs, as a rule, alternately in each of the two ovaries, once every four weeks during the thirty-five years of sexual life. A vast number of the Graafian follicles, after developing to a certain extent, undergo regressive changes, both during childhood and during adult life. The cellular elements degenerate, phagocytes wander into the follicle and attack the degenerating ovum, so that finally the follicle is replaced by connective tissue, without the formation of any corpus luteum.

The ovum, when set free, is directed into the open end of the Fallopian tube by the current set up by the cilia with which the epithelium is furnished. There may be other directive forces besides this, for, when the ovary on

* MARSHALL. *Q.J.M.S.*, 1904, 48.
† HEAPE. *Proc. Roy. Soc.*, 1905, **B.76**, 260.

one side is removed, and the Fallopian tube on the other side closed, pregnancy, though less likely to occur, is far from infrequent. According to Corner, the passage of the ovum along the Fallopian tube occupies three days.

After the discharge of the ovum, the remaining portions of the follicle undergo a characteristic series of changes, which results in the production of the *corpus luteum.* Immediately after the rupture, the cells of the membrana granulosa rapidly increase in size, a few of them undergoing mitotic division, so that a dense mass of cells is formed, nearly filling the original follicle. At the same time, the cells of the internal theca proliferate at the periphery, and the cells thus formed also grow in among the cells filling the Graafian follicle. The luteal cells finally attain a size four or five times that of the cells of the membrana granulosa in the mature follicle. Blood vessels grow from the external theca towards the centre of the follicle. The corpus luteum (Fig. 643) attains its greatest size in the human about the nineteenth day of the menstrual cycle, at which stage it has a grey colour: it then gradually undergoes regressive changes. The cells within the follicle then undergo fatty degeneration and present a yellow colour, due to a lipochrome known as *lutein.* If, wever, the ovum, which has been discharged, undergoes fertilisation, and pregnancy results, the corpus luteum continues to grow for a considerable time and attains its largest size at about the third month, though it persists, often in an apparently degenerating condition, until after the end of pregnancy. The big corpus luteum found in pregnancy is often spoken of as the 'true' corpus luteum, and is distinguished from the *corpus luteum spurium* of menstruation, or of ovulation without fertilisation. There is no essential difference, other than that of size, between these two kinds of corpora lutea.

THE ŒSTROUS CYCLE

The sexual activity of females shows recurrent changes, which vary in detail according to species, the whole cycle being known as the œstrous cycle (Heape, Marshall). In some animals, known as monœstrous, such as the bitch, there is only one œstrous period in each sexual season, of which the bitch has two a year, and between the seasons a period of quiescence, or anœstrus, is found. In others, *e.g.* mare, pig, rat and mouse, there are several heat or œstrous periods in the season, and these are separated by short resting intervals called diœstrus. In the pig, there is, at intervals of three weeks, an œstrous period lasting three days. In the human subject there are no actual alternations of rest and activity. The phases of the œstrous cycle may be classified as follows:

(1) Pro-œstrus, the preparatory phase.

(2) Œstrus, the period of 'heat,' during which alone, in most animals, copulation is permitted.

(3) Metœstrus, or luteal phase.

(4) Diœstrus (or anœstrus), sexual rest.

These periodic modifications of sexual activity are accompanied by changes in the generative organs, in general metabolism and activity (œstrus being accompanied by increase of general activity), as well as in the behaviour towards the male, and these have been extensively studied during the last few years.

The recurrent changes in the generative organs are normally associated with ovarian changes (follicular ripening and corpus luteum formation; they affect the external genitalia, the vagina, uterus, and often (especially during

metœstrus) the mammary glands, and these changes are closely related to the normal sexual life in any given species. It is simplest to regard the œstrous cycle as made up of a basic *follicular phase*, consisting of a short diœstrus, a pro-œstrus and an œstrous period, with a short transition phase of met- or post-œstrus ; and such a basic cycle is well seen in the unmated mouse. In most other animals, and in the mated mouse, the cycle is further modified by a *luteal phase*, due to the greater development of the corpus luteum, which, in the unmated mouse, is small and transient. As the luteal phase is con-

A B

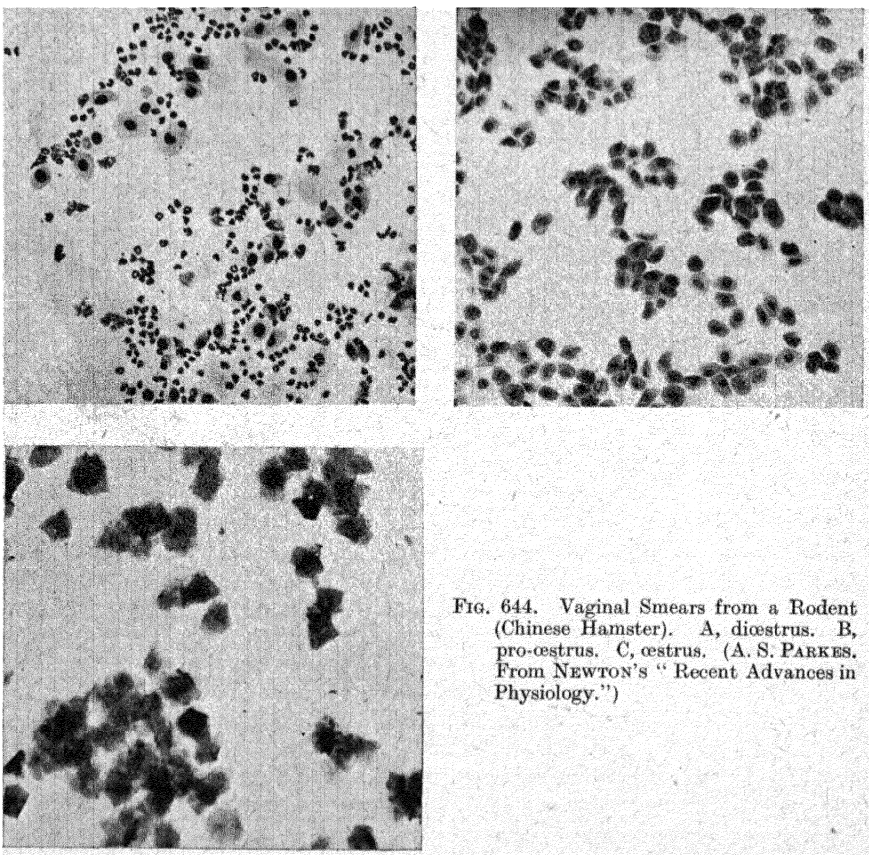

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FIG. 644. Vaginal Smears from a Rodent (Chinese Hamster). A, diœstrus. B, pro-œstrus. C, œstrus. (A. S. PARKES. From NEWTON'S "Recent Advances in Physiology.")

nected with the functions of the corpus luteum, and may be regarded as a phase superadded to the basic follicular stage, and showing a good deal of species variability, we shall consider it later.

During pro-œstrus, growth of the follicle occurs, and at œstrus, in most animals, maturation and ovulation follow, though in some, such as the rabbit, this only happens if and when copulation takes place.

The study of the œstrous cycle has been greatly facilitated by the discovery, made by Stockard and Papanicolaou,* that the vaginal lining of some mammals undergoes characteristic histological changes during each phase.

STOCKARD and PAPANICOLAOU. *Amer. J. Anat.*, 1917, **22**, 225.

In the rat and mouse, for example, in pro-œstrus the epithelial layers increase in number and in thickness, and then in œstrus the superficial cells lose their nuclei, become keratinised (cornification) and desquamate. Afterwards a transitory infiltration with leucocytes leads on to a return of the epithelium to a resting state, with few layers. Hence, by microscopical examination of vaginal smears at regular intervals, the state of the cycle at any time can be determined.

These changes, as seen in the smear, are briefly as follows (Fig. 644). In the pro-œstrous period there are nucleated epithelial cells only; during œstrus the vagina is cornified, during metœstrus leucocytes appear in the smear, during diœstrus there are leucocytes and nucleated epithelial cells. The whole cycle in the unmated mouse takes about five days (Parkes), occupied approximately as follows: Pro-œstrus, eighteen hours; œstrus, forty-two hours; metœstrus, twelve hours; diœstrus, two and a half to three days.

In the stages of pro-œstrus and œstrus the uterus becomes enlarged and congested and filled with fluid, which may be blood-stained. Its contractions become slow, large and strong, as contrasted with the feeble but frequent contractions in diœstrus or anœstrus.

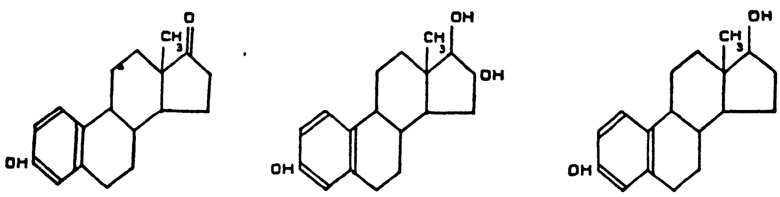
The Causation of Œstrus. If both ovaries are removed the œstrous cycle ceases, either at once or after one more period. The œstrous cycle also ceases during pregnancy and in some animals during lactation. It is not the maturation of the Graafian follicles that is the prime mover in bringing about the condition of œstrus, since Parkes has shown that in mice the œstrous cycle persists as in the normal animal, after the total obliteration of the follicular system by exposure to X-rays, either before, or at any time after, birth.* It is clear, therefore, that although the presence of the ovary is essential, the presence of the ripening follicles is not. According to many investigators the essential source of the phenomena is the theca interna of follicles of all sizes. This is further borne out by the observation that in those cases where there was one last œstrous period following double oöphorectomy, the follicles of the ovaries removed had not yet reached maturity. This shows that, instead of ovulation being the cause of œstrus, they must both have some common cause, and there is clear proof that this is the gonadotrophic anterior pituitary hormone.

ŒSTROGENS. It was clearly shown in 1923 by Allen and Doisy † that an œstrus-producing substance was present in the liquor folliculi. This, on injection into oophorectomised mice, brought on the state of œstrus. Substances having this action are now called *œstrogens* or *œstrogenic substances.* An œstrogenic substance can be extracted from the solid parts of the ovary, from the placenta, from the urine of both men and women, but in larger amount from the urine of women at about the time of ovulation, and in still larger quantity from the urine of pregnant females. The substance is almost insoluble in water, but forms soluble alkali salts. When emulsified with water and administered subcutaneously to oophorectomised mice, œstrus is induced in the course of the second day. Œstrogens are recognised, and extracts containing them are standardised, by the use of this method.‡ A mouse unit is the amount of a substance required to cause cornification in ten out of twenty oöphorectomised mice (but some 200 such units are needed to induce copulation). Another test is that based on the increase in weight of the uterus of an immature mouse.

* PARKES. *Proc. Roy. Soc.*, 1926, **B.100**, 172; 1927, **B.101**, 71, 421; **B.102**, 51.
† ALLEN and DOISY. *J. Am. Med. Assoc.*, 1923, **81**, 819.
‡ MARRIAN and PARKES. *J. Physiol.*, 1929, **67**, 389.

The discovery by Aschheim and Zondek (1927) that large amounts of œstrogen were present in the urine of pregnant women soon led to the isolation of one of them in a pure crystalline form. This was achieved almost simultaneously by Doisy,* Butenandt,† and Laqueur,‡ in 1929–30. The substance isolated proved later to be a hydroxyketone of the formula $C_{18}H_{22}O_2$.§ Shortly afterwards Marrian (1930)‖ isolated a second crystalline active substance which was a trihydroxy-substance. They are now called *œstrone* and *œstriol* respectively,¶ and can be separately estimated, when present together, by a colorimetric method.** Both compounds are present in the urine of normal women (100 to 600 units daily), and of men (20 to 100 units), but are present in large amount (150,000 to 500,000 units per day) in pregnancy urine in a combined form (as glucuronides) of low œstrogenic power, from which the free and active compounds are liberated by hydrolysis with acid. Œstrone has a higher activity when tested on adult ovariectomised mice, about twice that of the trihydroxy compound. Œstriol is the more active in the induction of precocious œstrus in immature animals. A third œstrogen is present in ovarian tissue, and is called *œstradiol*; it is the most active of them all, about six times the potency of œstrone. Which of the three compounds is the one actually elaborated by the ovary is not at present clear, though it has been supposed to be œstradiol, which is converted to the more soluble œstriol for elimination in the urine. The international units of œstrous activity are those of 0·1 μg. of œstrone or œstradiol monobenzoate (1 mouse unit = 0·06 μg. of œstrone).

Further work indicated the following formulæ for these substances :—



ŒSTRONE ŒSTRIOL ŒSTRADIOL

They are thus sterols, and closely related to some other reproductive hormones : *e.g.* progesterone and testosterone.

They are not species-specific. Œstrone when added to the isolated guinea-pig uterus reduces the effect of oxytocin, so that smaller contractions result from it : in the pregnant uterus also it modifies the powerful contractions produced by oxytocin, retarding their rhythm and facilitating relaxation between the contractions.††

These œstrogens can be administered by subcutaneous injection in dilute alcohol, or in oil, or by implanting the solid beneath the skin. They are much less effective by mouth. These three ovarian compounds do not complete the list of naturally occurring œstrogens, for others are found in some species, *e.g.* equiline and some others (all sterols) in the pregnant mare's urine. Also

* Doisy, Veler and Thayer. *Am. J. Physiol.*, 1929, **90**, 329.

† Butenandt. *Naturwiss.*, 1929, **45**, 879. *Deutsch. med. Woch.*, 1929, **55**, 2171. *Z. physiol. chem.*, 1930, **191**, 140.

‡ Dingemanse, de Jongh, Kober and Laqueur. *Deutsch. med. Woch.*, 1930, **56**, 301.

§ At various times the names "*folliculin*," "*menformon*" "*theelin*" and the "*a-hormone*" have been proposed for what is probably the same hormone.

‖ Marrian. *Bioch. J.*, 1930, **24**, 1021; *et al*, 1931, **26**, 25, 1932, **26**, 1233.

¶ Doisy has suggested the names "*theelin*" and "*theelol*" for these compounds.

** Cohen and Marrian. *Bioch J.*, 1934, **28**, 1603.

†† Marrian and Newton. *J. Physiol.*, 1932, **77**. Proc. 4.

numerous synthetic substances have œstrogenic properties; some of these are sterols and some are quite unrelated, *e.g.* the most powerful is diethyl dihydroxy-stilbene (or diethyl-stilbœstrol)* which is as potent as œstradiol, though not chemically similar.

The occurrence of œstrus in animals deprived of follicles by exposure to X-rays suggests that the hormone is normally produced in the stroma of the ovary. As regards its high concentration in the placenta, it is evident that, owing to the size of this organ, the placenta contains far more œstrogen than both ovaries; it has been suggested that the placenta takes up the hormones from the blood, and so protects the fœtus from their effects, but it seems more probable that they are formed in the placenta itself. Much of it is excreted in the urine of the mother.

The main effect of the injection of an adequate dose of œstrogen into the oöphorectomised female is the onset of the uterine and vaginal changes characteristic of œstrus, and also enlargement of the mammary glands. The uterus enlarges, becomes hyperæmic, and, it is stated, shows more powerful rhythmic contractions. The mammary glands (even in the male) show extension and branching of the ducts, but not of the secreting parts (Fig. 646). Large doses will check an already established lactation. Injection of œstrogen does not cause follicular maturation. The production of the hormone and ovulation, though due to a common stimulus, and normally coincident, are mutually independent.

The fate and interconversions of the œstrogens in the body are little understood.

Influence of Anterior Pituitary. If, as indicated above, the ripening of Graafian follicles is not the cause of the presence of œstrogens, how are we to explain the periodicity of œstrus, or the occurrence at puberty of the first œstrous cycle? The work of Smith and Engle,† and Zondek and Aschheim ‡ gives support to the view that the advent of puberty, as well as the rhythmicity of the œstrus phenomena, including the periodic ovulation, are part of an endocrine phenomenon, in which the anterior pituitary plays a determining *rôle*. This is effected by the gonadotrophic hormones, which have since been obtained in a state of partial separation from the other pituitary hormones. Injection of suspensions of the anterior lobe of the pituitary body into adult females is followed by immediate maturation of large numbers of ova, and formation of multiple corpora lutea, or, if pregnancy follows, by the formation of abnormally large numbers of fœtuses. Injected into immature animals, the suspension brings about precocious follicle ripening and œstrus, after a latent period of about three days, *i.e.* a day longer than is required for the effect of an injection of œstrogen to manifest itself. Injection into oöphorectomised animals produces no results, which makes it evident that the effects which are produced are due to an inductive action on the ovary. In partially oöphorectomised animals, however, the hypertrophy of the remaining ovarian tissue is greatly accelerated by administration of the anterior pituitary substance. Extirpation of the pituitary body leads to atrophy of the gonads and accessory organs or, in immature animals, to failure of maturity changes.

The pituitary is capable of stimulation by nerve impulses reaching it from the hypothalamus, *viâ* the stalk.§ Fee and Parkes || showed that, in

* Dodds, Lawson and Robinson. *Proc. R. S.*, 1939. **B. 127**, 140.

† Smith. *Amer. J. Physiol.*, 1927, **80**, 114; Smith and Engle, *Amer. J. Anat.*, 1927, **40**, 159.

‡ Zondek and Aschheim. *Klin. Woch.*, 1927, **6**, 248.

§ Brooks. *Amer. J. Physiol.*, 1938, **121**, 157.

|| Fee and Parkes. *J. Physiol.*, 1929, **67**, 383.

rabbits, removal of the pituitary prevents the normal ovulation following copulation.

Animals that have an anœstrous season in winter, *e g.*, ferrets, can be brought into œstrus by prolonged exposure to light. This response to light cannot be brought about in hypophysectomised animals * and is impaired by section of the optic nerves It is therefore concluded that the stimulation of the gonads is brought about by stimulation of the anterior pituitary from the retina In this reflex, the visual cortex and superior colliculi appear not to be involved, and the path seems to be either to the ventral nucleus of the lateral geniculate body, or to the subthalamus by way of the accessory optic tracts. But how the impulses then reach the anterior lobe is doubtful; it may be *viâ* the hypothalamus †

There appear to be two ' gonadotrophic ' principles formed in the anterior pituitary (Fig. 645): one, a water-soluble substance, called the *follicle stimu-*

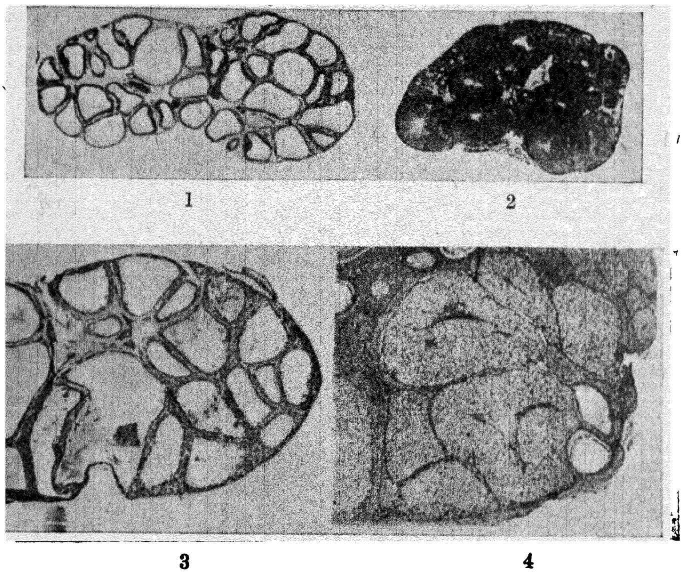


FIG 645. To illustrate the effect of the follicle-stimulating and luteinising hormones on immature ovaries

(1) Follicle stimulating effect on rat, from F.S.H. fraction of 1 g. sheep pituitary. No luteinisation

(2) Ovary of rat after unfractionated pituitary of sheep, 0·1 g. Full of corpora lutea.

(3) Rabbit, after F S.H. fraction of 2·5 g sheep pituitary.

(4) Rabbit, after 2·5 g. whole sheep pituitary.

(After HISAW *et al.*)

lating hormone (F.S.H.), stimulating follicular maturation, and one, insoluble in water, called the *luteinising hormone* (L.H.), provoking premature luteinisation ‡ ; these two hormones are normally excreted in small amounts in the urine. After oöphorectomy, or in states of hypo-functioning ovaries, such as at the menopause, the follicle-stimulating hormone is present in the urine in increased amount. Since in those cases the pituitary body has an increased content of the hormone and also contains numbers of large basophile cells, it is supposed that the hormone is formed in the basophile cells.

* HILL and PARKES. *Proc. R S*, 1933, **113B**, 537.

† LE GROS CLARK, *et al.* *Proc. R. S.*, 1939, **126B**, 449

‡ WALLEN-LAURENCE. *J. Pharm.*, 1934, **51**, 263 ; SMITH. *J. Amer. Med. Assoc.*, 1935, **104**, 553.

RELATION OF THE CORPUS LUTEUM TO THE CYCLE. The basal ovarian cycle is modified to a variable extent by the presence of the corpus luteum. This structure has functions specifically connected with pregnancy, apart from which condition its effects are only incidental. These effects are responsible for the *luteal phase* of the œstrous cycle. Thus, effects due to the corpus luteum in the unmated mouse are small, in correspondence with the insignificant development of the corpora formed after ovulation. After mating with vasectomised males, the corpora persist for a longer period and attain a greater development; the period of the cycle is then lengthened to about twelve days, during which time the uterus is much enlarged and vascularised, and there is pronounced growth of the mammary glands. This condition is called *pseudo-pregnancy*, and is seen as a normal sequel to the œstrous period in the bitch and other species where the corpora lutea of ovulation persist for a much longer time, irrespective of whether sterile copulation has occurred or not. In the bitch, the uterus is enlarged, with the endometrium thickened and vascularised, and there is enlargement of the mammary glands : as the condition passes off there is breakdown of the endometrium, with hæmorrhage. In normal pregnancy in all species, where the corpora lutea maintain a still greater development, the œstrous cycle is suspended and all the above phenomena are accentuated. Consideration of this and other facts indicates that the corpus luteum produces a hormone having the following functions :—

(1) Inhibition of œstrus and ovulation.

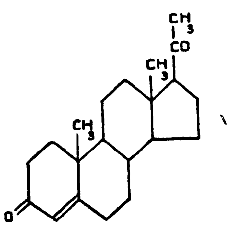
(2) Sensitisation of the uterus.

(3) Maintenance of pregnancy by improvement of the nutrition of the uterus.

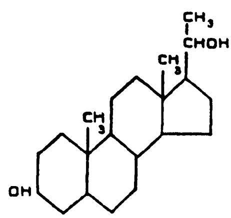
(4) Enlargement of the mammary glands during pregnancy and pseudo-pregnancy. Also, other special phenomena accompanying pregnancy, *e.g.* the remarkable relaxation of the pelvic ligaments which occurs in the guinea-pig, the mucification of the vaginal mucosa in the rat, modification of the endometrium in the rabbit, etc., etc.

Whether all these effects are to be attributed to the production, in the corpus luteum, of a single hormone, or of more than one, is as yet uncertain.

One substance has been isolated and identified with certainty.* Its structure is shown below :—



PROGESTERONE



PREGNANDIOL

and it has received the name *progesterone*. If progesterone is administered, it is converted into *pregnandiol*, the glucuronate of which is excreted in the urine.

The inhibition of œstrus and ovulation which is effected by the agency of the corpus luteum can be shown in a variety of ways. Thus, in the cow the removal of the corpora lutea of ovulation hastens the onset of the next cycle † : conversely, if luteal formation is augmented, as H. M. Evans found

* *Annual Rep. Prog. Chem.*, 1934, **31**, 209, 326.

† HAMMOND. The Physiology of Reproduction in the Cow. 1927. (Cambridge.)

to happen after injection of alkaline extracts of anterior pituitary, there is cessation of the œstrous cycles, together with other changes characteristic of pseudo-pregnancy.

Progesterone appears from the work of Frænkel to be essential for the preparation (sensitisation) of the endometrium for the attachment of the fertilised ovum, thus initiating pregnancy. It was shown by Loeb that, in the guinea-pig, when corpora lutea were present, mechanical irritation of the uterine mucosa, *e.g.* by a stitch passed through the uterus, led to local accumulation of decidual cells, which represented the attempted formation

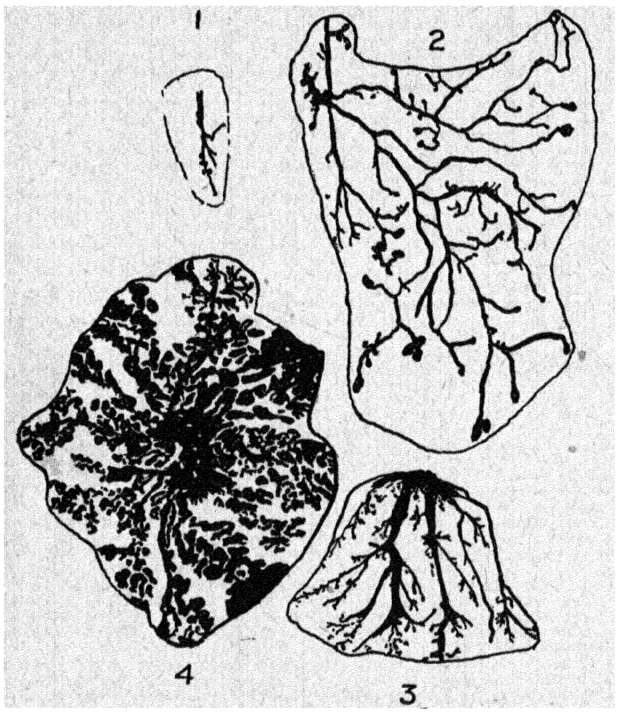


FIG. 646. Development of Mammary Gland by Ovarian Hormones.

(1) Mammary gland of male mouse (× 8).

(2) Mammary gland of male mouse after 4 rat units of œstrogen daily for thirty days. This corresponds to the development in a virgin adult female (× 4½).

(3) Mammary gland of spayed rabbit after twenty days' injections of progesterone, preceded by œstrogen treatment. No corpus luteum type of alveolar growth (× 1).

(4) Similar to (3), but œstrogen and progesterone were given together. Growth and development resembles that at mid-pregnancy. (NEWTON'S "Recent Advances in Physiology," after TURNER, 1932.)

of a placenta ; but after removal of the corpora lutea, this response vanished. Progesterone facilitates these progestational reactions in rabbits which have been oöphorectomised during œstrus, or which, after oöphorectomy, have received preliminary injections of an œstrogen, but otherwise does not cause them in oöphorectomised rabbits. This means that the uterine response is the result of the joint action of an œstrogen and progesterone.

Other actions of progesterone are an abolition of spontaneous contractions of the uterus, and, when œstrone is also given, a considerable growth of the mammary gland (Fig. 646) with extension of the actual secreting alveoli.

Even after the placenta has been fully formed, however, the corpus luteum

appears to be essential for the continuance of pregnancy, and removal of the corpora, or of the entire ovary, is followed in many species by abortion. But if progesterone is administered, oöphorectomy does not terminate pregnancy. As a still clearer demonstration that this effect of oöphorectomy in pregnancy is not due to the removal of other ovarian hormones, the following experiment by Parkes may be quoted. He destroyed the follicular tissue of one ovary, in mice, by X-rays, and the animals were then mated and became pregnant: when the normal ovary, which alone contained corpora lutea, was removed, abortion followed, although it was shown by separate experiments that the remaining X-rayed ovary was able to produce œstrogens.

MENSTRUATION

Puberty in the girl is marked by the onset of menstruation. Under this term is understood a flow of blood and mucus from the uterus, recurring about every 28 ± 3 days and lasting each time from four to eight days. Menstruation at intervals of about four weeks is a phenomenon which occurs in all the Old World primates. In some, including man, the only outward indication is the periodic bleeding: in others, *e.g.* macaques, baboons, there are areas of skin which become highly coloured during certain phases of the cycle, or at other times if œstrogen is injected.* Menstruation is suspended during pregnancy and lactation. The amount of blood lost at each menstrual period varies between 10 and 200 grammes. The flow is intermittent, and dependent on uterine contractions, and the blood is not coagulable and contains an anti-coagulant substance. Some toxic substance of unknown nature is said to be present in the sweat during menstruation. During the 'period' there are often disturbances of other functions, and a general disinclination for exertion. Before the first menstrual period, other signs of approaching sexual maturity are usually observed and are attributed to the elaboration, by the ovary, of an internal secretion. These include rapid growth, with changes in the skeleton, leading to the typically feminine pelvis, a development of the mammary glands, and the growth of hair on the pubes. At the same time, there is development of the mental characteristics typical of the sex.

CHANGES IN THE UTERUS. In describing the menstrual cycle, it is customary to number the days from the commencement of one flow to the commencement of the next.

Rhythmic contractions can be recorded at all stages by suitable small balloons passed into the non-pregnant human uterus.† In the first half of the cycle they are small and frequent; in the latter half, stronger but less regular. During menstruation strong waves occur at intervals of two to three minutes (Figs. 647, 648). Vasopressin causes spasmodic contraction of the uterus at all stages of the cycle, but oxytocin has no action at all on the non-pregnant uterus,† nor in early pregnancy.‡

The histological changes§ are briefly as follows: at the first day the mucosa, or endometrium, is 1 mm. thick and the glands are short simple tubules, between which are fusiform stroma cells. This may be called the *basal zone*. Preparation for menstruation begins at once, and the *phase of regeneration* occupies till the fourth day. It consists in the appearance of a new superficial layer, of epithelium, covering up the bare stroma, and about 0·5 mm. thick. Then follows the *phase of proliferation* up to the fifteenth

* PARKES and ZUCKERMAN. *J. Anat.*, 1931, **65**, 272; ZUCKERMAN, *B.M.J.*, 1932 (ii.), 1093; The Social Life of Monkeys and Apes. 1932. (London: Kegan Paul.)
† MOIR. *Edin. Med. J.*, 1934, **41**, 93.
‡ ROBSON. *J. Physiol.*, 1933, **79**, 83.
§ SHAW. *J. Physiol.*, 1925, **60**, 193; BARTELMEZ. *Contrib. to Embryol.*, No. 142, 1933; Carn. Inst. Wash. Publ. 435.

day; in this the superficial layer reaches about 3·5 mm. in thickness, with nuclear division in the gland cells, growth and secretion of the tubules and swelling of stroma cells. The enlargement and secretory activity of gland tubules increases until the twenty-eighth day (*phase of secretion*), when the œdema of the stroma becomes localised to the middle zone of the endometrium, and the capillaries become dilated. From the twentieth to the twenty-eighth day, the progestational changes become more apparent: the superficial zone, with close-packed stroma cells, closely resembles the compact layer of the decidua, while the middle zone, with dilated glands and œdematous stroma, resembles the spongy layer. The total endometrium is now about 6 mm. thick. When the flow begins, the *phase of desquamation* (twenty-eighth to second days), these two outer zones become disorganised, infiltrated with leucocytes and phagocytes, and ultimately are shed, with some bleeding, leaving the basal zone containing the lower ends of the glands, with the stroma bare. After the flow the epithelium from these glands grows over the bare stroma to form a lining (one to four days) and the next cycle has begun (Figs. 649, 650).

Similar histological changes have been seen to occur in small pieces of surviving monkey's endometrium transplanted into the anterior chamber of the eye.* After preliminary vasoconstriction, subepithelial hæmorrhages occurred and small papillæ

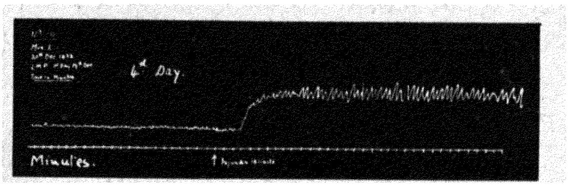


FIG. 647. Contractions of Non-pregnant Human Uterus on fourth day of Cycle, showing small rapid Contractions and Response to 10 Units of Pituitary Extract. (After MOIR.)

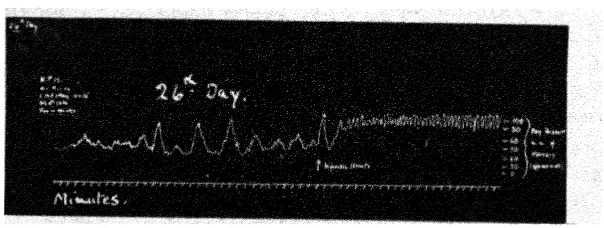


FIG. 648. Larger Contractions of Non-pregnant Human Uterus on twenty-sixth day of Cycle, showing Response to 10 Units of Pituitary Extract. (After MOIR.)

formed on the free surface. These ruptured and bled for about half an hour, after which new papillæ formed and ruptured. Desquamation began locally after the first day.

The Causation of Menstruation.† There is no doubt that menstruation normally depends on rhythmical activity of the ovary, in discharging œstrogens and progesterone at appropriate times. This in its turn depends on the output of gonadotrophic (follicle-stimulating) hormone from the anterior pituitary. Its onset coincides with the first production of ripe ova in the ovary, and it ceases, with the cessation of ovulation, at the menopause. In cases where the ovaries have been removed before puberty, menstruation never occurs. Removal of both ovaries during adult life brings about a premature menopause.

Despite its cyclic character the menstrual rhythm cannot be spoken of as 'œstrus.' The female may experience desire at any period of the cycle, and though coitus may be refused during menstruation itself, or for some time

* MARKEE. *Anat. Rec.*, 1933, **55**, 66.
† ALLEN. *J. Amer. Med. Assoc.*, 1935, **104**, 1901.

after, this is on æsthetic grounds rather than from absence of desire. Evidence as to the relation of ovulation to menstruation rests chiefly on observations made on the ovaries in abdominal operations (Shaw *) and by Corner, Hartman, and others, on menstruation in monkeys. These tend to show that the whole cycle may be roughly divided into a ' follicular ' (œstrogen)

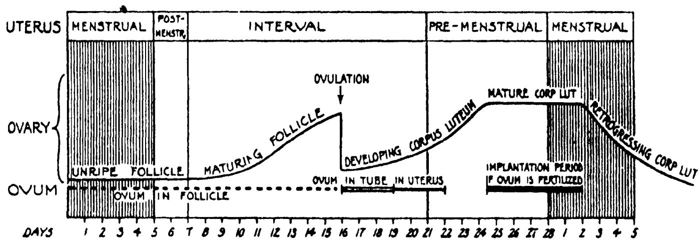


FIG. 649. Course of Events in the Human Menstrual Cycle. (G. W. CORNER.)

phase leading up to ovulation, *i.e.* the first fourteen to sixteen days, and a ' luteal ' (progesterone) phase for the remainder. It seems probable that the ripening and discharge of the ova in the human ovary occur about the thirteenth to seventeenth day of the menstrual cycle. In fact, live human ova have been found in the Fallopian tubes on the fourteenth day of the cycle.† Œstrogen excretion rises to a peak (600 units daily) at about mid-cycle. In the baboon, the lively colour of the sexual skin, due to processes accompanying the maturation of the ovum, abruptly subsides at about the

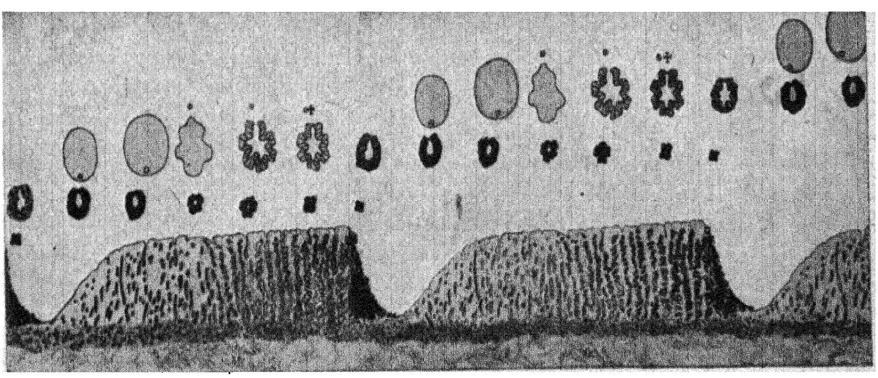


FIG. 650. Diagram showing, from left to right, the Changes in the Human Endometrium (below) and Ovary (top row, this month's follicle or corpora lutea ; lower row, last month's) during two complete Menstrual Cycles. (After SCHRÖDER.)

mid-point of the cycle and begins to appear again shortly after the onset of menstruation. It can be produced artificially by injection of an œstrogen. During the follicular phase, and as a result of the effect of œstrogen, there is growth of the endometrial glands. According to Frænkel, after ovulation, which normally takes place once in each menstrual cycle, the corpus luteum at once begins to develop and attains maturity in about five days, *i.e.* about the twenty-first day. It persists in this state until the commencement of the

* SHAW. *J. Physiol.*, 1925, **60**, 193.
† ALLEN *et al.* *Contrib to Embryol*, 1930 (No. 414), **22**, 45.

menstrual flow, when it usually abruptly degenerates; in any case, it is already undergoing degeneration by the end of the flow. The excretory derivative of progesterone, pregnandiol glucuronate, is found in the urine from the middle of the cycle (forty-eight hours after ovulation) until just before menstruation begins.* If progesterone is administered to a hysterectomised woman, no pregnandiol is excreted, whereas a normal woman would excrete about 45 per cent. of the progesterone in this form. It is therefore supposed that progesterone is converted into pregnandiol glucuronate in the uterus.

Coincident with the growth of the corpus luteum, and the presence of progesterone in the blood, the uterine glands undergo rapid growth and the mucosa undergoes changes preparatory to receiving a fertilised ovum. This, the progestational stage, thus corresponds to the pseudo-pregnancy stage of the lower mammals. If impregnation occurs, the embryo becomes embedded in the thickened membrane, and this continues to grow and to form the placenta, which involves the opening of maternal blood-vessels into large intervillous spaces. Several theories regarding the significance of menstruation have been advanced. These need not be discussed here, but according to the most popular one it may be regarded as due to failure of fertilisation to take place. If the ovum is not fertilised, the corpus luteum undergoes retrogression, and, possibly in consequence of this, the hypertrophied mucosa breaks down, the *débris* being discharged, together with extravasated blood, as the menstrual fluid. In conformity with this, excision of the copora lutea at once precipitates a menstrual flow.

It would seem that two, if not three, factors are concerned in the causation of normal menstruational changes, of which the menstrual flow is the final stage. For the proper growth changes in the endometrium, œstrogen and progesterone appear to be essential; for the causation of the menstrual flow withdrawal or reduction in both hormones is probably necessary.† First, there is the follicular phase in which the uterus and the endometrial glands enlarge and become highly vascular. This, the proliferative phase, can be imitated by the injection of large doses of œstrogen into oöphorectomised monkeys. Stoppage or great reduction of the dose of œstrogen leads to a menstrual flow in about a week. Secondly, there is the luteal phase, in which the endometrium undergoes the characteristic changes. This phase can be imitated on spayed monkeys by following up the œstrogen injections by injections of progesterone, beginning at the fourteenth day, when the œstrogen dose is reduced. Lastly, there appears to be a phase which is responsible for the actual necrosis of the prepared endometrium; this may be imitated, in the experiment quoted above, by stopping the progesterone after ten days, *i.e.* at the twenty-fourth day, as a result of which bleeding begins about the twenty-eighth day.

A difficulty in all theories of menstruation which cannot be ignored is the unquestionable fact that normal menstruation without ovulation or corpus luteum formation often occurs in apes, and sometimes in women. In this case we assume that there have been no progestational changes in the endometrium, and that menstruation is due merely to cessation of œstrogen.

The probable course of events in the human menstrual cycle is summarised in the diagram above (Fig. 649). The changes in the endometrium and in the ovary are shown diagrammatically in Fig. 650.

* VENNING and BROWNE. *Endocrinol.*, 1937, 21, 711.
† PARKES. *B.M.J.*, 1931 (ii.), 559.

THE MENOPAUSE

At the menopause, ovarian function ceases. The condition normally appears more or less gradually, and the symptoms vary in severity, but it may be artificially induced by surgical removal of both ovaries, or by destruction of the ovaries by deep X-ray or radium treatment, and then the symptoms are more severe.

Cessation of menstruation may be sudden, or may be preceded by irregularity. The breasts, uterus and external genitalia atrophy and sexual desire ultimately wanes. These phenomena are to be attributed to the reduction or loss of ovarian hormones. Aspects of virilism become apparent, such as growth of hair on the face, and an alteration of the features, and these have been said to be due to overaction of the suprarenal cortex. The anterior pituitary may become overactive too, since there is an increase in follicle-stimulating hormone (C.U.) in the urine, or even signs of incipient acromegaly. The thyroid activity is lessened, and definite hypothyroid symptoms are often encountered.

Changes in the circulation, such as hot flushes or palpitation are common. Perhaps most striking, though most often concealed, are mental and emotional unstability which may be long-lasting and may go on to clear-cut insanity.

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THE MALE REPRODUCTIVE ORGANS

In all the higher animals, we may divide the male reproductive organs into the essential organs, which form the germ cells, the spermatozoa, and the accessory organs, which have as their office the facilitation of the access of the spermatozoa to the ova (fertilisation). We also have to consider male secondary sexual characters, *e.g.*, the comb of the fowl, the horns of the stag, and, in man, the body build and stance, the deep voice, and the specialised distribution of hair on face, chest and pubic region.

The essential sexual organ of the male is represented by the testis. This is made up of a collection of convoluted tubules, the seminal tubules, which are contained in a number of compartments separated by fibrous septa. The tubules present few or no branches, each one being about 500 mm. long. Several tubules unite to form a straight tubule, which leads by a series of communicating spaces, the rete testis, into the vasa efferentia (Fig. 651). These join to form the duct of the epididymis, coiled into a mass lying at the back of the testis. The epididymis is composed of the convolutions of this single duct, which is about 20 feet long. From the lower end of the epididymis, the vas deferens, a tube with thick muscular walls, leads by the abdominal ring to the base of the bladder, where it opens into the beginning of the urethra in its

prostatic part. Just before it joins the urethra as the ejaculatory duct, each vas deferens is joined by a diverticulum, the seminal vesicle, which lies along, and is attached to, the base of the bladder. The seminal vesicles are lined by columnar epithelium, and have plain muscle in their walls. They contain a glairy liquid which may, but which usually does not, contain some spermatozoa. The prostate itself, which surrounds the first part of the urethra, is composed of a matrix of unstriated muscular fibres, enclosing numerous branched racemo-tubular glands. These open by numerous ducts into the prostatic urethra, and mostly near the entry of the ejaculatory ducts. A blind recess from the prostatic urethra, called the utriculus, is a vestige of the Mullerian duct, which, in the female, forms the Fallopian tubes, uterus and vagina. From the point of entry of the vasa deferentia to its orifice, the urethra represents a common passage for the urine and semen. It passes, therefore, through tissues forming the penis, which is especially adapted for the introduction of the semen into the female. In the urethra we distinguish the prostatic, the membranous and the penile portions. Into the beginning of the penile portion, the bulb of the urethra, open the ducts of the two glands of Cowper. In the penis itself, the urethra is surrounded with erectile tissue,

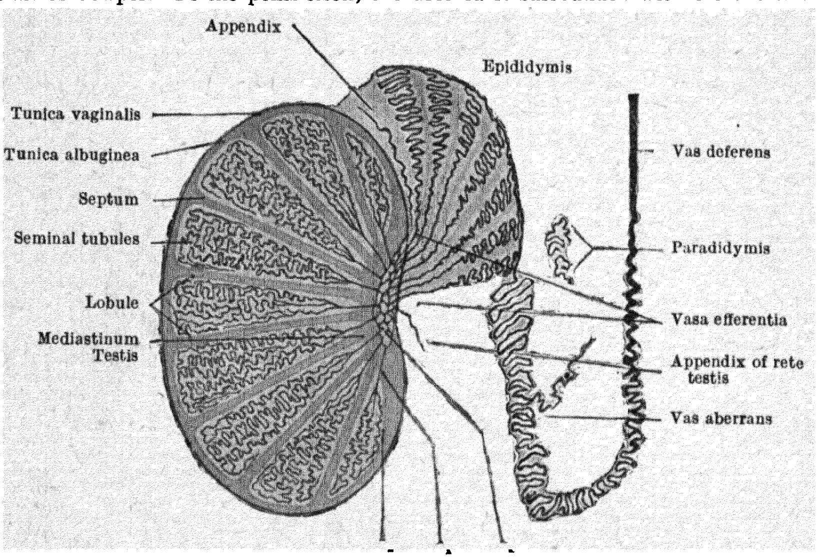
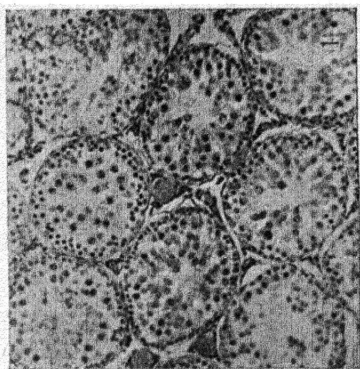


FIG. 651. Diagrammatic Representation of the Course of the Seminal Tubules in the Testis and Epididymis. (After NAGEL.)

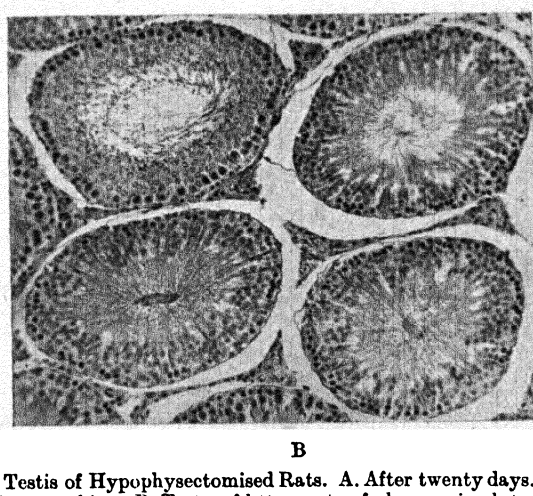
forming the corpus spongiosum, and lies between the two corpora cavernosa, which consist of the same kind of tissue. The erectile tissue is a spongy meshwork of elastic and unstriated muscle fibres, enclosing spaces in free communication with the efferent veins of the organ. The arterioles also open into these spaces, but under normal circumstances both the arterioles and the muscle tissues of the framework are contracted, so that the blood trickles very slowly from the arterioles into the spaces, whence it escapes readily by means of the veins. If these muscle fibres be relaxed, so that the blood can pass rapidly into, and distend, the spaces, the tissue swells and becomes harder, causing ' erection ' of the organ.

Puberty and Spermatogenesis. In the immature testis, *i.e.* from birth up to puberty, the seminal tubules are filled with cells having large nuclei. Some of these are the spermatogonia, the mother cells of the future spermatozoa, while the others form the cells of Sertoli, whose function it is to act as nurse cells to the developing spermatozoa. In hypophysectomised animals the testes remain in, or revert to, an immature condition. The actual formation of spermatozoa begins at puberty, and can be produced precociously (or in a hypophysectomised animal) by the ' follicle stimu-

lating' hormone of the anterior pituitary (Fig. 652) which, as we have seen, is also responsible for the maturation of ova in the female. Since this hormone has a stimulating action on the production of gametes of both sexes, it is therefore often known, perhaps more appropriately, as the *gametokinetic hormone*; other pituitary hormones cause hypertrophy of the interstitial cells of the testis, and this tissue then produces male sex hormones which cause growth and development of the various accessory organs of reproduction.



A



B

FIG. 652. Testis of Hypophysectomised Rats. A. After twenty days. No treatment. Tubules atrophic. B. Testis of litter mate of above animal, treated for twenty days by injection of pregnancy urine gonadotrophin. The animal was mated, and fertile, on the tenth and twentieth days. (After SMITH and LEONARD.)

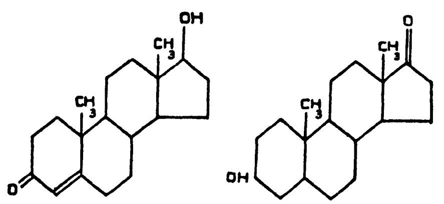
In the formation of spermatozoa, the spermatogonia divide many times to form the spermatocytes, which in their turn undergo reduction division to form the spermatids, as already described. By a further division and modification of the latter, the fully developed spermatozoa are formed. These, when mature, pass by the tubules of the testis into the epididymis, where they are stored until an ejaculation takes place, or failing that, die and are reabsorbed. Their movement is probably facilitated by the cells lining the tubule of the epididymis, as well as by the secretion of the lining membrane of the seminal vesicles. The spermatozoa are motionless while in

the seminiferous tubules of the testis, but become actively motile in the vas deferens, or when mixed with prostatic secretion. They can then travel, head foremost, at about 20μ per second (70 mm. per hour). Spermatozoa recovered from the female tract soon after their introduction are always in active motion. This is soon lost, however, in most species, including man (probably in thirty to forty hours), and fertilising power is lost sooner still. The formation of the spermatozoa is continuous, though the rate at which this occurs is variable and is regulated by the sexual activity of the individual. Spermatozoa survive longest at neutral reactions and low temperatures ; on being warmed to body temperature the activity is increased, but the length of survival shortened. In many mammals, including man, the optimal temperature for spermatogenesis is below the normal body temperature ; hence if the testis fails to descend into the scrotum, where a lower temperature is maintained, it becomes non-functional.

The Semen. In the fully formed semen, the spermatozoa originating in the testis are mixed, not only with the fluid secreted by the lining membrane of the epididymis and of the seminal vesicle, but also with the viscous secretions of the prostatic glands and of Cowper's glands. Nevertheless, it contains spermatozoa in enormous numbers, the semen emitted at a single act of coitus (2 to 4 c.c.) usually containing 200 to 300 million spermatozoa.* Though the majority of these are probably capable of fertilising an ovum, this act is carried out by only one. There is no relation between numbers of spermatozoa and volume of ejaculate, nor between spermatozoa motility and age of the man. Frequent ejaculations may considerably reduce the numbers of spermatozoa in the semen.

It is probable that in ejaculation the secretion of the Cowper's glands, with which the urethra is already filled, is expelled first ; then follows the alkaline prostatic secretion mixed with increasing numbers of spermatozoa and finally the viscous secretion of the seminal vesicles. In the mouse and some other animals this last secretion is coagulated by a vaginal enzyme to form a solid vaginal plug which prevents loss of semen.

THE MALE HORMONES. Between the seminiferous tubules are groups of so-called *interstitial cells*, which appear to have an important relation to the sexual life of the individual. Removal of the testes before puberty prevents the development of the accessory organs, prostate, seminal vesicles, Cowper's gland and penis, and of the secondary sex characters ; ligature of the vasa deferentia, which is followed by complete degeneration of the seminiferous tubules, but not of the interstitial cells, does not have these consequences. Hence it is assumed that the development of these characters is to be ascribed to the formation, by the interstitial cells, of an internal secretion having that influence on the individual. This is supported by the observation that when testis grafts are made into castrated animals, the male secondary sex characters develop in the usual way. Masculinisation of ovariectomised females has also been claimed to occur when testis substance is grafted.



TESTOSTERONE ANDROSTERONE

Androgens. An androgen is a substance which causes the development of male organs and characters. It is a parallel in the male of the œstrogen

* Hotchkiss and Brunner. *Amer. J. Med. Sci.*, 1938, **196**, 362.

in the female. A hormone having these effects has been extracted from testes ; it is called *Testosterone*, and a similar substance, called *Androsterone*, is excreted in small amounts in the urine, of both sexes, but more in male

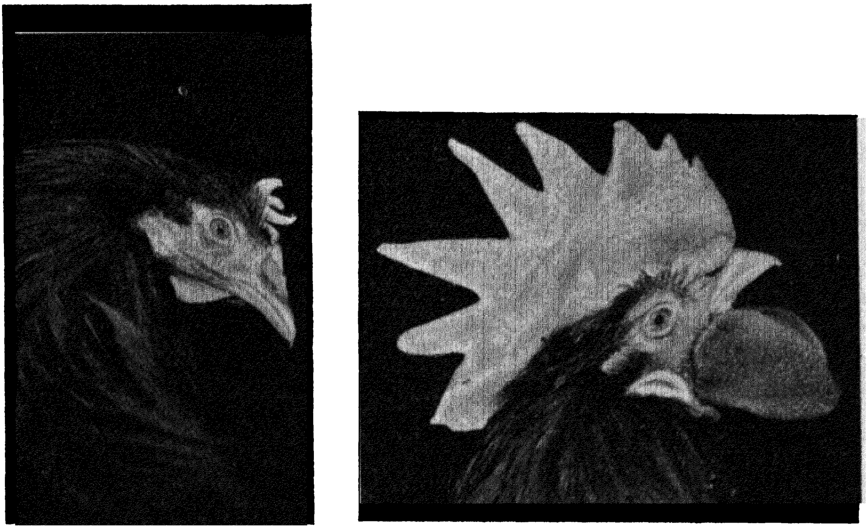


FIG. 653. Effect of an Androgen on a Capon's Comb. On left, the capon. On right, the same after twenty-two daily injections of androsterone. (After PARKES, *Bioch. J.*, 1935, **29**, 1422.)

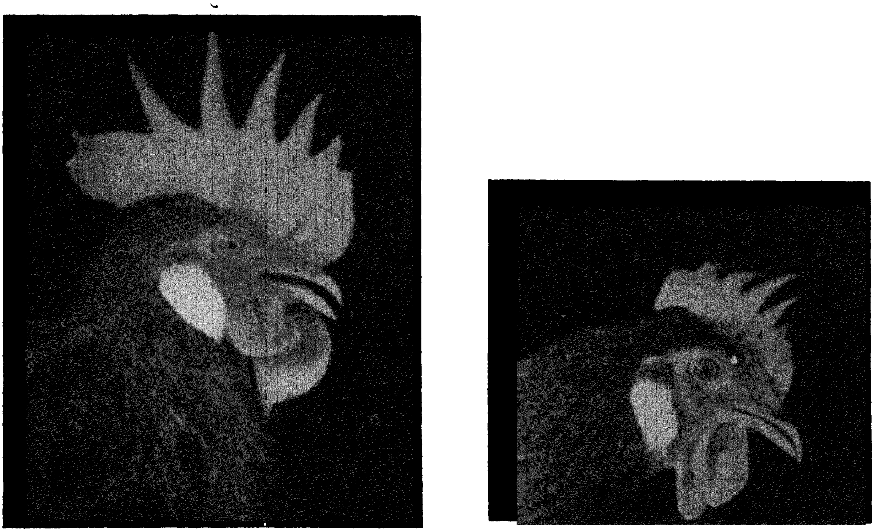


FIG. 654. Effect of an Œstrogen on a Cock. *Left*, before, and *right*, after implantation of tablet of 65 mg. of œstrone. (After EMMENS, *J. Physiol.*, 1939, **95**, 386.)

urine (5 mg. daily), from which it has been isolated by Butenandt,* Schoeller and others. The crystalline materials are hydroxy-ketones, insoluble in water, which have been made synthetically and are closely related to the

* BUTENANDT. *Z. f. angew. Chem.*, 1931, **44**, 905.

female œstrogens.* Many other substances also have androgenic activity. It should be noted that there is no sharp line of demarcation between androgens and œstrogens, each being often capable of producing the effects

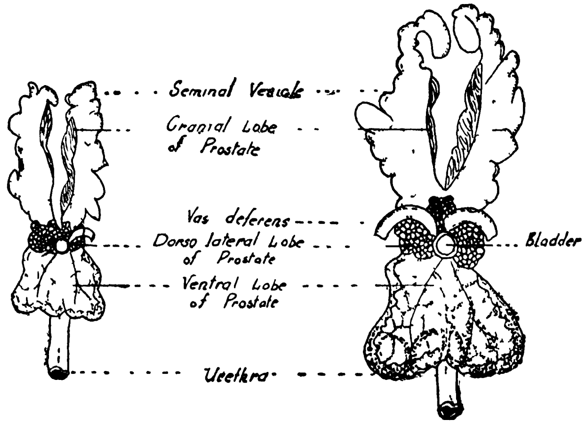


FIG. 655. Internal Genitals of two young Rats of same Litter, showing effect of Androgens. (Drawn by G. Brownlow.) (GADDUM. "Pharmacology," Oxford Press, 1940.)

of the other if given in large doses. Yet the effects of an androgen are in many ways antagonistic to those of an œstrogen (Figs. 653, 654). Among the physiological effects produced is a growth of the comb in capons † and it also prevents the atrophy of the prostate, seminal vesicles (Figs. 655, 656),

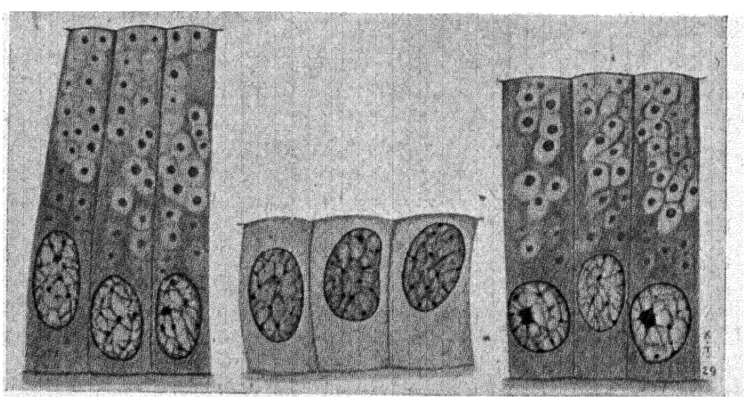


FIG. 656 Effect of Castration and of Androgen on the Epithelium of the Seminal Vesicles. (1) Cells from normal animal, showing secretion granules; (2) cells from twenty-day castrate: (3) cells of twenty-day castrate treated with an androgen. (Newton's "Recent Advances," after MOORE, HUGHES and GALLAGHER.)

Cowper's glands, vas deferens, etc., of castrated rats.‡ Assay of androgens can be made by measurement of the growth caused in the comb of the capon (castrated cock) (Fig. 653), or by the increase in weight of the prostate and

* *Ann. Rep Progress of Chem.*, 1934, **31**, 323.
† McGEE *et al.* *Amer. J. Physiol.*, 1928, **87**, 406; BLYTH, DODDS and GALLIMORE, *J. Physiol.*, 1931, **73**, 136.
‡ MOORE. *J. Amer. Med. Assoc*, 1935, **104**, 1405.

seminal vesicles of immature rats (Fig. 655). The international unit is 0·1 mg. of androsterone.

In man and various other 'constant breeders,' *e.g.* rats and guinea-pigs, the hormone is constantly produced from puberty onwards throughout sexual life; it is not stored, but regularly escapes in the urine. Hence atrophy of the prostate, etc., commences in a few days if castration is performed. The rate at which the hormone is produced depends on the state of nutrition and general health, but it ceases at once if the anterior lobe of the pituitary body be removed. This indicates that the anterior pituitary provides a hormone which provokes the production of androgens, and further proof of this is shown by the fact that the testicular function may be restored to hypophysectomised males by injection of fresh anterior pituitary or of the F.S.H. (gametokinetic) fraction. Conversely, androgens, and perhaps other substances of testicular origin, can inhibit the production of the gametokinetic hormone by the pituitary.

In those animals such as squirrels, which show seasonal sexual activity, the anterior pituitary seems to be non-functional during the resting phases, and this is probably the case also in all males prior to puberty.

A female hormone, or *œstrogen,* is also present in male urine. This hormone, when injected into males, leads, in some species, to hypertrophy of the prostate; in others to epithelial growth and even cornification in the utriculus, together with diminution of prostatic gland substance and increase in its plain muscle stromata.* It is tempting to suppose that the somewhat analogous changes in the senile enlarged prostate have as their source an alteration in the normal balance between male and female hormones. The enlargement caused by an œstrogen can be inhibited by an androgen.

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DEGREE OF SEX-SPECIFICITY OF HORMONES

The sex hormones are not completely sex-specific, *i.e.* the hormones which characterise one sex are not absent from the other, nor without action on it. In the case of the pituitary hormone, we have seen that the substance which stimulates the growth and development of the gonads is probably the same in both sexes, and for that reason is sometimes called the gametokinetic hormone. The gonadotrophic hormones of pregnancy urine (P.U.), of pregnant mare's serum (P.M.S.) and of castrate urine (C.U.), not only cause follicle stimulation or luteinisation, but also have effects on the testis, causing it to increase in weight, by growth of the interstitial cells (by P.U. and C.U.) or of the seminiferous tubules (by P.M.S.).

As regards the œstrogens and androgens, substances of both types are present in the urines of both sexes, though usually the androgen predominates in male urine and the œstrogen in female urine. Moreover, androgens when injected into females may produce two kinds of effect. One of these is a tendency to masculinisation, *e.g.* there is a growth of the clitoris, a masculine type of growth, and some of the male secondary characters may appear, *e.g.* growth of hair on the face in women. The female cycle may

* Parkes and Zuckerman. *Lancet,* 1935, **228,** 924.

also be suppressed, perhaps as a result of an inhibitory effect on the pituitary gonadotrophic hormone. The other effect may be œstrogenic in nature, *e.g.* growth of the uterus, or duct development of the mammary gland.

The administration of œstrogens to males, especially to castrates, may similarly produce feminisation, as seen in growth of the mammary glands and nipples, and growth of the prostate, utriculus masculinus, and seminal vesicles, accompanied by cessation of the secretory activity of these organs. But androgenic effects can also be obtained; these are best seen in the reinforcement of the effects of testosterone when given together with an œstrogen. Progesterone also has effects on the development of the male mammary glands, which resemble those on the female.

The explanation of these common properties is no doubt to be sought in the chemical relationship between these hormones, and is confirmed by the fact that suprarenal cortical hormones, which are also nearly related chemically, can in large doses produce both androgenic and œstrogenic effects.

COPULATION AND IMPREGNATION

The spermatozoa, stored in the epididymis and vas, are introduced into the female genital tract by the act of copulation, or coitus. After their entry they may ultimately come in contact with, and fertilise, the ovum, discharged from the ovary by bursting of a Graafian follicle. Before coitus can occur erection of the male organ must take place. The mechanism of erection is twofold. The most important factor, as was shown by Eckhard and Lovén, is an active dilatation of the vessels of the penis, especially of the smaller arteries. If the penis be cut across while in the flaccid condition, venous blood merely trickles away from the cut surface, whereas, if erection be excited, the flow of blood from the cut surface is increased eight to ten times, and the blood becomes bright scarlet in colour. A second factor is the contraction of the *ischio-cavernosus* or *erector penis* muscles, certain fibres of which pass over the dorsal veins of the penis and compress these vessels when they contract. Since ligature of the veins coming from the penis does not produce erection, the contraction of this muscle must be regarded as simply aiding the effects of the arterial dilatation. It is thus possible to excite erection in an animal, in which the second factor has been abolished by paralysing the muscles by means of curare.

Before, or at the beginning of, coitus analogous changes occur in the female organs, leading to erection of the clitoris and of the erectile structures of the vulva. The glands of the vulva, especially the glands of Bartholin, secrete a mucous fluid, thus lubricating the passage into the vagina. The friction between the glans penis and the wall of the vagina ultimately causes a reflex discharge of motor impulses in both male and female (the 'orgasm'). In the former, the muscular walls of the vasa deferentia and seminal vesicles enter into rhythmic contractions, thus ejaculating the spermatozoa and secretions they contain into the urethra. The spermatozoa, mixed with the secretions of the epididymis, the seminal vesicles, the prostatic glands, and the glands of Cowper, form the semen, which, at the orgasm, is ejaculated from the urethra by rhythmical contractions, from behind forwards, of the bulbo- and ischio-cavernosi muscles.

The exact physical events of the female orgasm are uncertain. It has been stated that movements take place coincidently in the uterus, so that its axis more nearly corresponds to that of the vagina, and that the os uteri dilates. The female orgasm, however, does not play an essential part in

impregnation, which, as shown by Spallanzani in the eighteenth century, can be effected by artificial insemination, *i.e.* injection of semen into the vagina by a syringe. It is improbable that any material is aspirated into the uterus during coitus, as was at one time supposed. A more important factor is probably the movement of the spermatozoa themselves.* As we have already seen, these are introduced into the female passage in countless numbers. They will be chemiotactically attracted by the alkaline mucus, secreted by, and filling, the cervix of the uterus. Spermatozoa move, in a straight line, at the rate of 1 to 3 mm. per minute. Thus they might traverse the distance of 16 to 20 cm. between the os uteri and the trumpet-shaped orifice of the Fallopian tubes in three-quarters of an hour. But direct injection into the uterus would shorten this time greatly, and in animals spermatozoa have been found at the peritoneal end of the Fallopian tubes within fifteen minutes after coitus. Although ripe sperms remain viable for weeks within the epididymis, their life is brief once they have been introduced into the female tract, where few survive more than a day or two. Fertilisation of the ovum normally occurs in the Fallopian tube, and the fertilised ovum is then carried slowly down the tube into the uterus, partly by peristaltic contractions, partly by ciliary action of the tubal mucosa.

Nervous Mechanism of Coitus. Although, in both sexes, coitus is attended by a high degree of psychical excitement, yet its reflex part can be carried out when all impulses from the higher centres are cut off by section of the cord in the dorsal region. The centres presiding over the reflexes are situated in the lumbar and sacral spinal cord (ejaculation and erection respectively). The external generative organs, like the bladder, are supplied from two sets of nerve fibres—from the lumbar nerves through the sympathetic, and from the sacral nerves.

The fibres from the lumbar nerves arise, in the cat, from the second, third, and fourth, or the third, fourth, and fifth lumbar nerve roots, and, in the dog, from the thirteenth thoracic, and the first to the fourth lumbar roots. They run in the white rami communicantes to the sympathetic chain, whence they may take one of two paths.

(*a*) The great majority of the fibres run down the sympathetic chain to the sacral ganglia, whence fibres are given off in the grey rami communicantes to the sacral nerves; their further course is by the pudic nerves.

(*b*) A few fibres go by the hypogastric nerves to the pelvic plexus.

Excitation of these fibres causes contraction of the arteries of the penis, and of the unstriated muscles of the tunica dartos of the scrotum. In animals which possess a retractor penis muscle, excitation of the lumbar nerves causes strong contraction of that muscle. The effect of stimulation of these nerves is, therefore, to inhibit erection.

The fibres from the sacral nerves can be divided into two classes—visceral and somatic. The visceral branches run in the pelvic nerves, or *nervi erigentes*. Stimulation of these fibres produces active dilatation of the arteries of the penis or vulva, and also inhibition of the unstriated muscle of the penis, of the retractor muscle of the penis, when present, and of the vulva muscles. The somatic branches supply motor nerves to the ischio- and bulbo-cavernosi, as well as to the constrictor urethræ. In the female, they supply the analogous muscles, namely, the erector clitoridis (ischio-cavernosus) and the sphincter vaginæ (bulbo-cavernosus). Both sets of sacral

* PARKER. *Phil. Trans. R.S.*, 1931, 219.B. 381; CARLETON and FLOREY, *J. Obstet. & Gynæc.*, 1931, 38, No. 3.

fibres are therefore involved in the erection of the generative organs which accompanies coitus.

The internal organs, *i.e.* the uterus and vagina in the female, and vasa deferentia, seminal vesicles, and uterus masculinus in the male, differ from the external organs in receiving no efferent nerve fibres from the sacral nerves.* They are supplied with fibres, which pass out through the anterior roots of the third, fourth, and fifth lumbar nerves (in the rabbit and cat), and run through the sympathetic to the inferior mesenteric ganglia, whence they proceed by the hypogastric nerves. On stimulating these fibres, two effects are produced on the uterus and vagina, namely, a contraction of the small arteries, leading to pallor of the organs, and a strong contraction of the muscular coats.† In the vagina, the contraction can usually be seen to start from one end and spread to the other. The whole then remains for a time in a state of powerful tonic contraction, which affects both longitudinal as well as circular muscles. There is also secretion of Bartholin's glands.‡ In the male, stimulation of these nerves excites contraction of the whole musculature of the vasa deferentia and seminal vesicals, which may be strong enough to cause emission of semen from the penis. These effects on the uterus and seminal vesicles are not abolished by injection of atropine.

The ovary in monkeys has a sympathetic nerve supply from the tenth thoracic to seventh lumbar levels of the spinal cord.§

Removal of the sympathetic system causes impotence in males since ejaculation fails ; it has no effect on the œstrus cycle, course of pregnancy or parturition in females, || though in some of these lactation is inhibited.

The course of the sensory fibres which represent the afferent side of the arc, from the generative organs to the lumbo-sacral cord, has not yet been fully made out, but it seems probable that it corresponds to the course taken by the efferent fibres.

An accessory genital muscle, the *retractor penis*, is found in the dog, cat, horse, donkey, hedgehog (not in the rabbit or man). It consists of a thin band of longitudinally arranged unstriated muscle, which is inserted at the attachment of the prepuce, and is continued backwards to the bulb, where it divides into two slips which pass on either side of the anus. A few striated fibres are found in the posterior part.

The *motor* fibres to the muscle are derived from the lumbar sympathetic (*i.e.* the upper set of nerve roots), and run to the muscle in the internal pudic nerve. The pelvic nerves, on the other hand, carry *inhibitory* impulses to the muscle, thus enabling the concomitant vascular dilatation to take effect in producing erection of the penis.

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PREGNANCY

Fertilisation of the ovum takes place in the Fallopian tube. In the human subject, fertility is probably highest about a week or ten days after the end of menstruation and lowest about a week before its onset. The data published by Siegel,¶ in which the leave periods of German soldiers during the

* LANGLEY and ANDERSON. *J. Phys.*, 1895, **19**, 122

† Under some circumstances, stimulation of the sympathetic nerves may cause *relaxation* of the uterus, as also may the administration of adrenaline.

‡ BARRINGTON. *Int. Mtschr. Anat. Physiol.*, 1913, **30**, 1.

§ ZUCKERMAN. *Anat. Rec.*, 1934, **58**, Sup. 43.

|| CANNON *et al.* *Amer. J. Phys.*, 1929, **89**, 83 ; BACQ, *Amer. J. Phys.*, 1932, **99** 444.

¶ SIEGEL. *Münch. med. Woch.*, 1916, **63**, 748.

last war were correlated with the incidence of pregnancy in their wives, generally supports this view. More recently, deliberate records of dates of menstruation and of acts of intercourse place the high fertility period at the tenth to thirteenth days.*

Directly one spermatozoon has penetrated into the ovum, a membrane is formed round the yolk, which prevents the entrance of any other spermatozoa. The fusion of the male and female pronuclei is followed immediately by division of the fertilised ovum, so that, by the time it arrives in the uterus (about eight days after fertilisation), it consists of a mass of cells known as the morula. At this time, the ovum has a diameter of about 0·2 mm. Pregnancy in the human being lasts about 280 days, *i.e.* ten periods, after the last menstrual period.

With the arrival of the fertilised ovum in the uterus, extensive changes begin in this and in the neighbouring organs of generation. The virgin uterus is pear-shaped, and its cavity amounts to about 2–5 c.c. Just before birth, the volume of the uterus is about 5000–7000 c.c., and the walls of the organ are thickened in proportion. In the hypertrophy of the uterine wall, all its elements are involved, but especially the muscle cells. There may be an actual formation of new muscle fibres, and each fibre grows in length and thickness, becoming finally between seven and eleven times as long, and three to five times as thick, as in the unimpregnated uterus (Fig. 657). There is at the same time a great growth of the blood vessels, which have to supply, not only the growing wall of the uterus, but also by means of a special organ—the placenta—the nutritional needs of the developing fœtus. The increase chiefly affects the veins, which thus become capable of holding a considerable store of blood; the enlargement begins early in pregnancy, and while the fœtuses are still very small, continues more slowly while the fœtuses grow rapidly, and finally, just before delivery, again increases quickly.† The rate of blood flow through the uterus increases at about the same rate as does the uterine blood content.‡

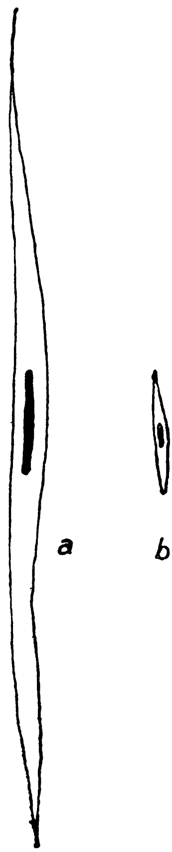

Fig. 657. Isolated Muscle Cells from the Uterus, showing the Hypertrophy during Pregnancy.

a. Fibre from uterus in ninth month of pregnancy.
b. Fibre from a non-gravid uterus. (After Bumm.)

Changes in the Uterine Mucous Membrane. After conception, the uterine mucous membrane shows a continuation of those hypertrophic changes which we have seen to occur regularly during the premenstrual phase (Fig. 658). Within fourteen days, it has attained a thickness of $\frac{1}{2}$ cm., and by the end of the second month $\frac{3}{4}$ cm. On section, it shows a compact layer, lining the cavity of the uterus, and outside this, abutting on the muscular tissue, is a spongy layer three times as thick as the compact layer. The superficial epithelium becomes flattened, loses its cilia, and degenerates. In the spongy layer, the uterine glands proliferate, the stroma cells are enlarged, and the blood capillaries are widely

* Pryde. *B.M.J.*, 1941 (i), 12.
† J. Barcroft and Rotschild. *J. Physiol.*, 1932, **76**, 447.
‡ J. Barcroft, Herkel and S. Hill. *J. Physiol*, 1933, **77**, 194.

dilated. The stroma cells become converted into the large *decidual* cells. By the time the fertilised ovum arrives in the uterus, the process of hypertrophy of the layers of the mucous membrane has already made some progress. As it lies on the mucous membrane, the outermost cells of the developing ovum exercise a destructive influence on the adjacent cells of the mucosa, apparently through some sort of digestion, so that the ovum sinks into the membrane and reaches the sub-epithelial connective tissue. Round the margins of the depression which the ovum has made for itself, the mucous membrane grows over the protruding part of the ovum (Fig. 659). When this has taken place, the different parts of the mucous membrane receive different names. Since (in man) they are all to be cast off with the fœtus at birth, each part is spoken of as a decidua, that lining the main body of the uterus being known as the *decidua vera*, that

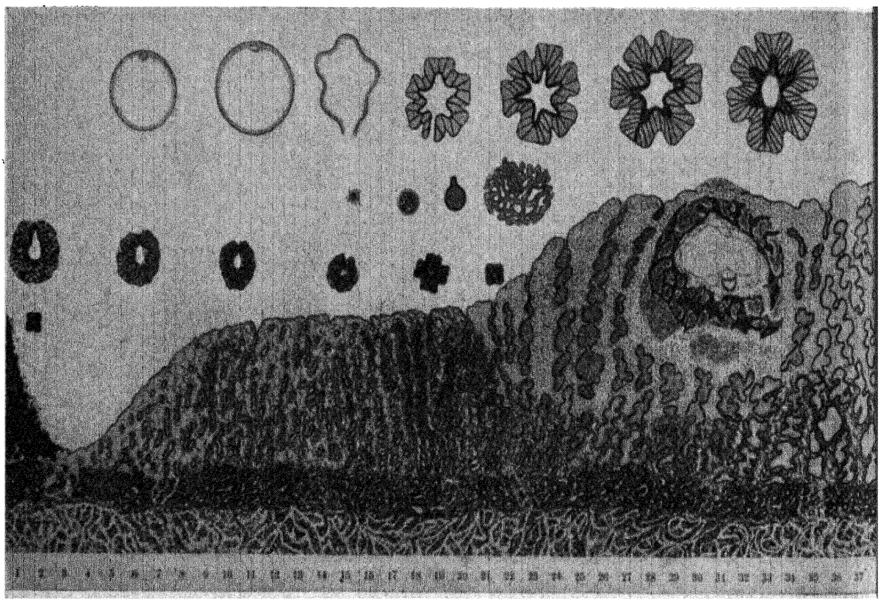

FIG. 658. Diagram showing Changes in the Human Endometrium and Ovary during the first 37 days of Pregnancy. (After SCHRÖDER.)

covering the protruding part of the egg as the *decidua reflexa*, while that to which the egg is immediately attached is the *decidua serotina* or *basalis*. It is from the latter that the placenta is formed. By the end of the second week, the blood vessels in this situation are considerably enlarged. This enlargement proceeds, affecting especially the capillaries and veins, until these form venous sinuses at the junction between the mucous membrane and the muscular coat. Changes take place at the same time in the embryo. When it sinks into the mucous membrane it has a diameter of 1 mm. The blastoderm is fully formed, with its three layers; the yolk sac, the body cavity and the amnion are present. The outermost layer of the epiblast becomes specially modified to serve for the nutrition of the embryo, and gives rise to numerous villi, the chorionic villi, so that the whole ovum has a shaggy appearance. Since this tissue takes no part in the further development of the embryo, but serves simply for its nutrition, it is often spoken of as the *trophoblast*. With the formation of fœtal blood vessels, these penetrate

into the villi, together with mesoblast. The villi grow into the venous spaces, especially in the basal part of the decidua, so that, at this period, the fœtal villi are immersed in maternal blood, the fœtal blood vessels being separated from the maternal blood by a double layer of epithelium, one layer of which is maternal and the other fœtal in origin. Later, these cells become reduced to a single layer.

Hormone Factors. Pregnancy is accompanied by pronounced changes in the amount and distribution of various sex hormones. Even in the early stages there is an excretion in the urine of a gonadotrophic hormone similar to, but not necessarily identical with, those of the anterior pituitary, viz. the follicle-stimulatory and the luteinising hormones. It is called the *pregnancy gonadotrophic substance* (P.U.). It reaches its maximum at about

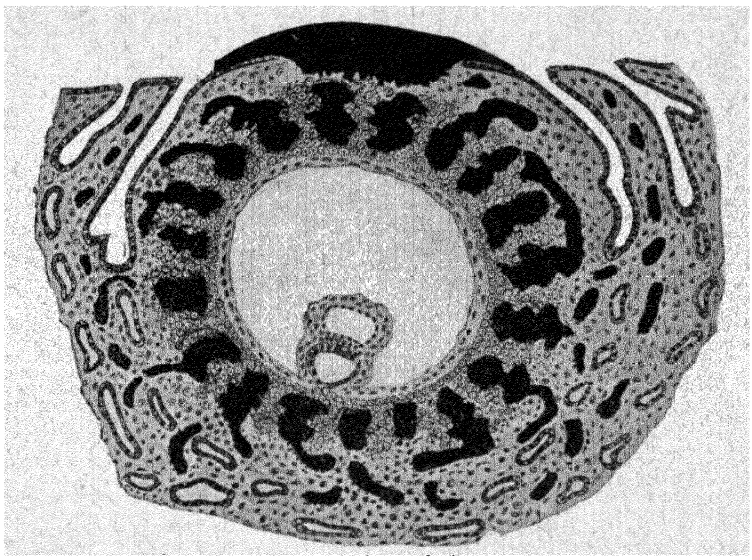

FIG. 659. Diagram to illustrate the Imbedding of the Ovum in the Decidua, and the First Formation of the Fœtal Villi in the form of a Syncytial *Trophoblast* (derived from the Outer Layer of the Ovum) which is invading Sinus-like Blood Spaces in the Decidua. (After T. H. BRYCE.)

the eighth week, falls till the tenth or eleventh week to a lower level, then stays constant till delivery, and disappears a few days later.

This discovery by Aschheim and Zondek * that a substance having gonadotrophic actions can be extracted from the urine of pregnant women, has led to the introduction of a valuable test for pregnancy.† To 1 to 3 c.c. of urine, previously extracted with ether, glucose is added ; it is then injected twice daily in various doses into immature female mice of 6 to 8 grammes body weight. Within forty-eight to seventy-two hours, the ovaries show follicular maturation, and at least one out of five mice shows the positive reaction—viz., one or more corpora lutea or blood points (hæmorrhagic follicles). It was at first thought that pregnancy urine contained two gonadotrophic hormones. The hormone causing precocious œstrus was given the provisional name Prolan A, and that causing luteinisation and formation of hæmorrhagic follicles Prolan B, but in spite of the similarity of their actions it

* ASCHHEIM and ZONDEK. *Klin. Woch.*, 1928, 7, 1404.
† ZONDEK. *Klin. Woch.*, 1931, 10, 1484.

is established that they are not identical with the anterior pituitary hormones. If given to hypophysectomised rats the action is only luteinising; the follicular maturation seen in immature animals being secondary to an effect on their own pituitaries. The P.U. principle is thus mainly luteinising in its action. It is assayed by studying vaginal cornification. The serum of pregnant mares (P.M.S.) contains both follicle-stimulating and luteinising hormones; the former can be assayed by finding the increase produced in the weight of the ovaries of immature animals.

A third gonadotrophic principle (C.U.) is found in the urine of ovariecto-

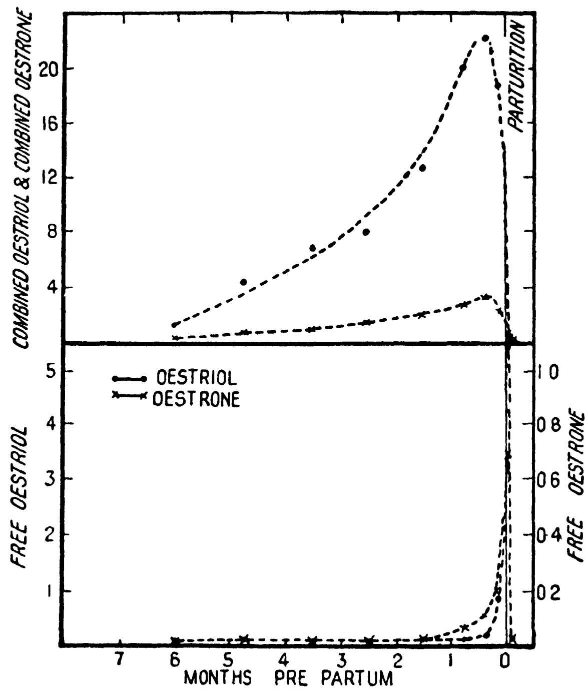

FIG. 660. Excretion of Œstrogen in the Urine during Pregnancy. Œstriol and Œstrone in free and combined forms are given in mg. per 24 hours. (COHEN, MARRIAN and WATSON. *Lancet*, 1935 (i.), 674.)

mised women, and after the menopause. It is mainly follicle-stimulating and may be a true pituitary hormone.*

International standards containing arbitrary fixed amounts of active principle have been laid down for the gonadotrophic luteinising substance of human urine of pregnancy (' chorionic gonadotrophin ') (0·1 mg.) and for the (follicle-stimulating) gonadotrophic substance of pregnant mare's serum (0·25 mg.). These standards are kept by the Medical Research Council.†

After the eighth week also, œstrogen in combined form (as a glucuronide) is excreted in increasing amount right up to the end of term.‡ The enlargement of the corpora lutea, and the increase in excretion of pregnandiol glucuronide in the urine from the eighth week onwards, shows an increasing formation of progesterone. The combined action of the œstrogens and progesterone

* BROWNE. *Can. J. Res.*, 1933, **8**, 180.
† *Bull. Health Organisation, League of Nations*, 1939, **8**, 884, 898.
‡ COHEN and MARRIAN. *Bioch. J.*, 1936, **30**, 57.

brings about enlargement and differentiation in structure of the mammary glands, and uterus, and other changes already referred to. Œstrogens can in large doses suppress lactation, but in small doses encourage growth of the mammary duct system and may sometimes stimulate the pituitary anterior lobe to produce prolactin.* As soon as labour sets in there is a sudden drop in the excretion of the combined and relatively inactive urinary œstriol and œstrone, and a sharp rise in the free and active forms (Fig. 660).†

FUNCTIONS OF THE PLACENTA. Besides playing an important part in the nourishment of the fœtus, it now seems almost certain that the placenta is a site for production of all the important hormones of pregnancy. The P.U. factor is almost certainly produced by chorionic tissue, since it is also excreted in such conditions as chorionepithelioma, in which there is no fœtus. In some species, including the human, pregnancy is not terminated by oöphorectomy, after which œstrogens and pregnandiol glucuronide continue to be excreted. Lastly, Newton has shown ‡ in the mouse, that if the fœtuses are destroyed, the placentæ remain, œstrus is suppressed, the mammary glands develop (independently of the ovaries), and finally the placentæ are delivered at the normal term.

Nutrition of the Embryo. At the earliest period of its development, the fertilised ovum is dependent for its nourishment on the remains of the cells of the discus proligerus adhering to it, or on the fluid of the Fallopian tube in which it is immersed. The first blood vessels which are formed serve to take up nourishment from the yolk sac. In man, this source of supply is insignificant, and, from the second week onwards, blood vessels traversing the chorionic villi come into close relation with the maternal blood, from which, henceforth, the whole growth of the fœtus is to be maintained by a special development of these connections in the placenta.

In the fully formed fœtus, blood passes from the fœtus to the placenta by the umbilical artery, and is returned by the umbilical veins. There is no communication between fœtal and maternal circulations. The placenta represents the fœtal organ for respiration, nutrition, and excretion. Thus, the umbilical artery carries to the placenta a dark, venous blood, which in this organ loses carbon dioxide and takes up oxygen, so that the blood of the umbilical vein is arterial in colour. The oxygen requirement of the fœtus is small, probably less than half of that of the same weight of maternal tissue. It is protected from all loss of heat, movements are sluggish, or for the most part absent, and the only oxidative processes are those required in the building up of the developing tissues. On the other hand, the fœtus has need of a rich supply of foodstuffs, which it must obtain through the placental circulation. It is imagined that the epithelium covering the villi represents a membrane through which pass the necessary foodstuffs and oxygen, from the maternal to the fœtal blood, and carbon dioxide, urea and other metabolic products in the reverse direction. The placenta contains a considerable amount of glycogen and fat, which may perhaps act as stores of material for the use of the fœtus in emergency.

As might be anticipated from their rapid rate of growth, the amount of blood in the uterus, placenta and fœtus rapidly increases during pregnancy; the fœtal goat, for instance, shows an increase of blood volume from about 16 c.c. at the sixty-eighth day to 300 or 400 c.c. at term. Expressed as a percentage of the weight of the fœtus, however, the amount falls slightly

* FOLLEY. *B.M.J.*, 1941 (i), 40.
† COHEN, MARRIAN and WATSON. *Lancet*, 1935 (i.), 674.
‡ NEWTON. *J. Physiol.*, 1935, **84**, 196; *Anat. Rec.*, 1938, **72**, 333.
§ ELLIOTT, HALL and HUGGETT. *J. Physiol.*, 1934, **82**, 160.

during pregnancy from about 25 to about 14 per cent., but if expressed as a percentage of the weight of fœtus plus placenta, the amount is more

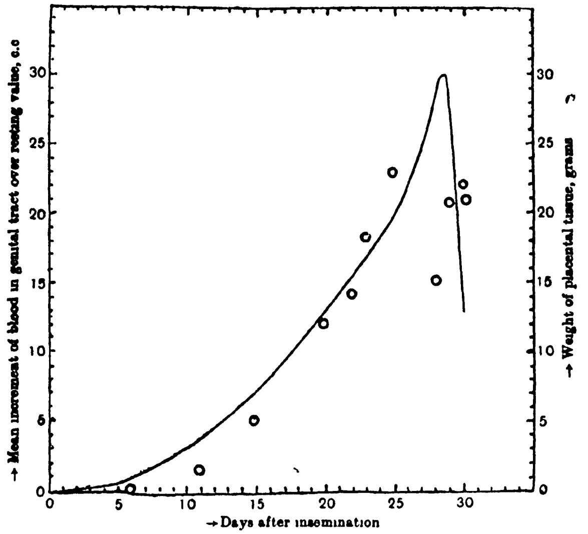

FIG. 661. Relation between Placental Weight (circles) and Volume of Blood in Uterus (line) in pregnant rabbit. (NEWTON'S "Recent Advances in Physiology," after BARCROFT and ROTHSCHILD.)

nearly constant at about 9 per cent. In other words, as pregnancy advances, the fœtus grows more rapidly than the placenta, but the blood volume roughly keeps pace with the growth of the combined uterine contents.

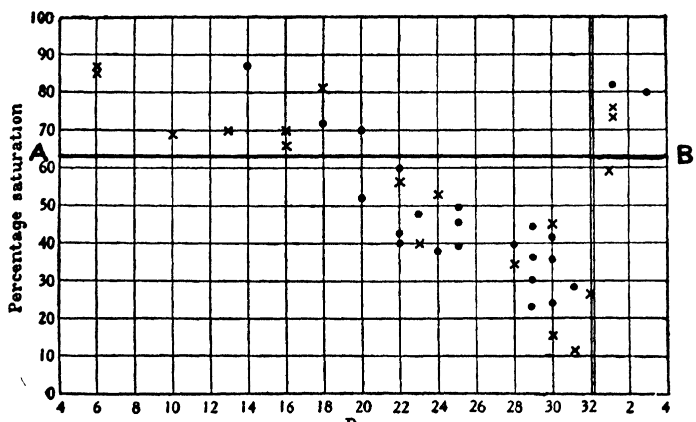

FIG. 662. Percentage Saturation with Oxygen of Maternal Blood leaving the Pregnant Horn of the Uterus at different stages of Pregnancy in the Rabbit. AB represents the average saturation of blood leaving the non-pregnant horn. Parturition on thirty-second day. (BARCROFT, FLEXNER, HERKEL, MCCARTHY and MCCLURKIN.)

Two factors are clearly of importance in facilitating the exchange between fœtal and maternal bloods, viz. the circulation rate in the fœtus, and the circulation rate in the maternal placenta. These have been estimated by Barcroft and his co-workers. In the fœtal sheep and goat, they found that

the output of the fœtal heart provides a fairly constant volume of blood per unit weight of fœtal tissue during the latter half of pregnancy, but that the volume per unit weight of whole tissue to be circulated, *i.e.* of fœtus plus placenta, steadily increases during the latter half of pregnancy, the volume per gramme per minute being about three times as much at full term as it was at about half term.

On the maternal side there is a great increase in the vascularity of the uterus, which, in fact, runs closely parallel to the increase in weight of the placentæ (Fig. 661). Since the growth of the fœtus soon outstrips that of the placenta, however, it would appear that the maternal blood circulation rate tends to lag behind the fœtal one. The relation between these two circulations becomes of paramount importance in connection with the supply of oxygen to the fœtus since, as pregnancy advances, the maternal venous blood leaving the placenta becomes less and less saturated with oxygen (Fig. 662).

The Fœtal Circulation. In the fœtus, from the middle of intra-uterine life, we find certain arrangements of the circulation which have long * been supposed to be directed to providing the heart itself, and the forepart of the body, especially the rapidly growing brain, with oxygenated blood, while the less important tissues of the limbs and trunk receive almost venous blood (Fig. 663). These old beliefs have been vindicated by direct experiment, in which radio-opaque solutions have been injected into the fœtal circulation, and X-ray cinematograph records taken.† As the arterial blood of the umbilical vein passes from the placenta, *viâ* the *ductus venosus*, into the inferior cava, it is mixed with the sluggish stream of venous blood, returning from the lower limbs and lower part of the trunk. Some of the umbilical vein blood, of course, can pass directly into the liver. The greater part of it, however, traverses the ductus venosus, to enter the inferior vena cava, by which it is carried to the right auricle. Here the bulk of it impinges on the Eustachian valve, and is directed thereby through the *foramen ovale* into the left auricle, whence it passes into the left ventricle, to be driven into the aorta. By the aorta, this partially arterialised blood is carried chiefly to the coronary vessels of the heart, and to the head and fore limbs. A small fraction of it, however, passes on through right auricle and ventricle to the pulmonary artery. The venous blood from the fore parts is also carried by the superior vena cava to the right auricle, right ventricle, and pulmonary artery. Of the but slightly arterialised mixture of bloods thus carried to the pulmonary artery, only a small part passes through the lungs, the greater part traversing the patent *ductus arteriosus* to be discharged into the descending aorta, whence about a third of it flows to the lower limbs and trunk, and two-thirds to the placenta by the umbilical arteries. In the fœtus, therefore, the work of the circulation is largely carried out by the right ventricle. The great thickness of the left ventricular walls, which is so characteristic of the adult, does not become evident until shortly before birth. The umbilical artery, accordingly, carries chiefly venous blood to the placenta, where it is arterialised by interchange with the maternal blood, and the arterialised blood is then returned to the fœtus by the umbilical vein—a state of affairs comparable with that resulting from passage of blood to and from the lungs of an air-breathing animal. Conversely, of course, the maternal blood conveyed to the uterus and placenta is arterial, and that returning is venous.

* SABATIER. Traité complet d'anatomie, 1791, ii., 493.

† BARCLAY, BARCROFT, BARRON and FRANKLIN. *Brit J. Radiol.*, 1939, 12, 505.

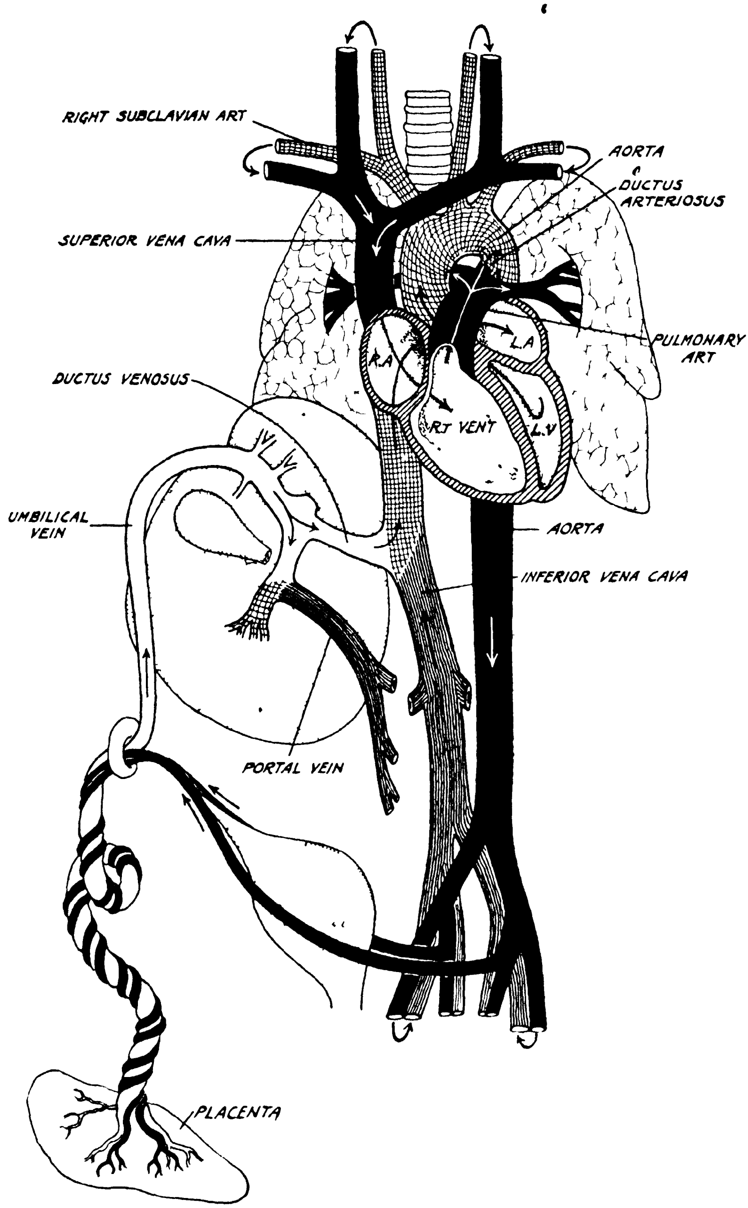

Fig. 663. Diagram of the Fœtal Circulation. (After Gray)

J. Barcroft found the following oxygen contents of the various fœtal bloods in the sheep :—

Blood of umbilical vein	= 79	per cent.	oxygen	saturation
,, thoracic inf. ven. cava .	= 56	,,	,,	,,
,, carotid artery . . .	= 41	,,	,,	,,
,, jugular vein . . .	= 33	,,	,,	,,
,, aorta (umbilical artery) .	= 23	,,	,,	,,

He believes that, as postulated, there is a crossing of the circulations, though not a complete one, and estimates that about two-thirds of the blood from

the "upper" circulation passes on to the lower one, while of the blood returning from the lower circulation two-thirds pass on to the upper.

SUPPLY TO THE FŒTUS. The rate at which oxygen is acquired by the blood circulating through the fœtal side of the placenta will depend especially on the relative rates of the fœtal and maternal placental circulations, and on the oxygen tensions on the two sides of the membranes. The most favourable conditions would be when the circulation rates on both sides were rapid, and the difference between the oxygen tensions of maternal and fœtal bloods great. Such information as we have is derived chiefly from the study of the rabbit, sheep and goat, and it is uncertain how far the conclusions drawn from these species can be transferred to man.

We have seen that, as pregnancy advances, the circulation rate on the

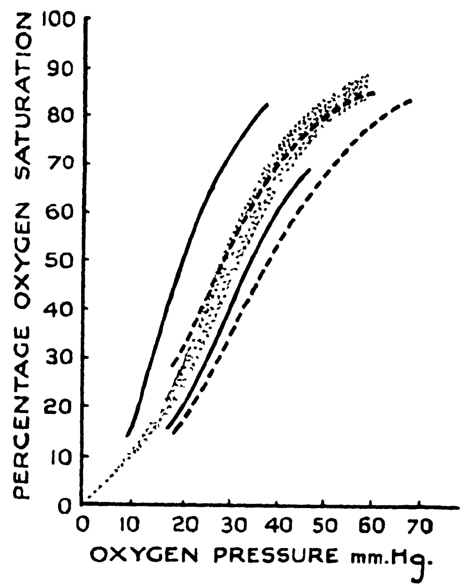

FIG 664 Oxygen Dissociation Curves of Fœtal and Maternal Goats The two left curves are from fœtal and the two right from maternal bloods. The shaded area covers the range of dissociation curves of normal adult goats Stage of gestation : continuous lines, 19½ weeks, interrupted lines, 14½ weeks. (NEWTON, "Recent Advances in Physiology," after BARCROFT and others. *Journ. Physiol.*, 1934, **83**, 192.)

maternal side fails to augment at the same rate as that on the fœtal side, which increases even more rapidly than does the weight of tissue to be supplied. In view of this it is not surprising to find that the percentage oxygen saturation of the blood leaving the uterus (much of which comes from the placentæ) steadily falls towards the end of pregnancy, when it may reach a very low figure, *e.g.* 20 per cent., as against about 65 per cent. saturation in the non-pregnant uterine venous blood, or 75 per cent. in the post-partum uterine venous blood.

The surprising fact which emerges from these results is that the fœtal blood coming from the placenta has so much greater a saturation with oxygen than has the maternal venous blood. Yet it has been shown that the gradient of tensions is such that the exchange can be accounted for by diffusion across the intervening membrane,* *i.e.* the *tension* in the fœtal blood

* HUGGETT. *J. Physiol.*, 1927, **62**, 373.

returning by the umbilical vein is *not* higher than that in the maternal venous blood. The key to this apparent discrepancy has been found by the study of the dissociation curves of fœtal and maternal bloods. It has been shown * that the curve of the fœtal blood is steeper than that for maternal blood, *i.e.* at a given oxygen tension the fœtal blood takes up more oxygen than does the maternal blood (Fig. 664). This appears to be partly because the maternal blood, owing to raised H-ion concentration, has a lowered oxygen affinity, but chiefly because the hæmoglobin of fœtal blood is different from that of the adult, in having a higher oxygen affinity.

Various relations are possible between the directions of flow in the maternal and fœtal vessels of the placenta, as suggested in Fig. 665. That which would give the highest oxygen tension in the fœtal blood would be as shown in A (the condition in the rabbit) in which the fœtal blood on leaving

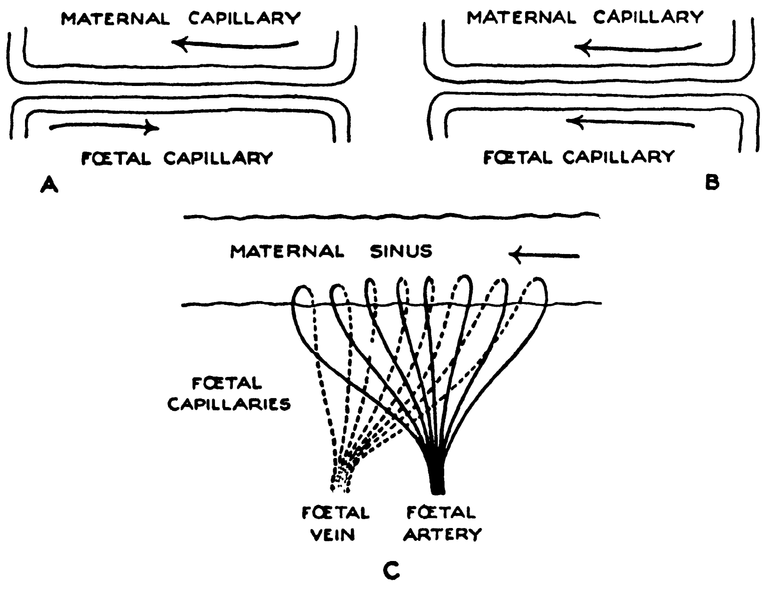

Fig. 665. Possible Relationships between Maternal and Fœtal Circulations. A, opposite flow; B, parallel flow; C, "perpendicular" flow. (NEWTON. "Recent Advances in Physiology.")

the capillary is in relation with the maternal blood just entering; in B, on the other hand, the fœtal blood would be in equilibrium with maternal venous blood. Probably in man the arrangement is as in C, which supposing that diffusion equilibrium were reached, would result in a fœtal oxygen tension in the umbilical vein blood higher than that in the maternal venous blood, but lower than that in the maternal arterial blood.

Actual determination of the oxygen saturation of the various bloods supports this conclusion. J. Barcroft found in the goat and sheep that the percentage saturations are somewhat as follows at about the middle of fœtal life:—

Arterial blood to uterus	95	per cent. oxygen saturation	Maternal circuit.
Venous blood from uterus	60	,, ,, ,,	
Umbilical artery (to placenta) . . .	15–45	,, ,, ,,	Fœtal circuit.
Umbilical vein (from placenta) . . .	70–80	,, ,, ,,	

* HASELHORST and STROMBERGER. *Ztsch. Geb. Gyn.*, 1930, **98**, 49; 1931, **100**, 48; BARCROFT *et al.* *J. Physiol.*, 1934, **83**, 192.

As regards the transfer of carbon dioxide, similar considerations will apply. There seems no reason to invoke any other aid than that of diffusion, for effecting the transfer from fœtal to maternal blood.

CHANGES AT BIRTH. The first breath taken by the newborn child is due to the effect of slight, or sometimes definite, asphyxia, consequent upon separation of the placenta from the uterus and to a lesser extent to the influence of cold and of drying of the skin. With sufficiently advanced fœtuses, opening of the uterus or of the uterus and sac in air leads to respiration, but opening under warm saline solution does not. Ligation of the umbilical cord or of the arteries supplying the uterus invariably causes respiration.* Respiratory movements, probably also asphyxial in origin, always occur late in intra-uterine life, but the amniotic fluid thus aspirated into the lungs is reabsorbed. The respiratory centre of the fœtus acts less readily than that of the adult animal.† This appears to be due to an inhibitory influence exerted by the forebrain.

As the time for birth approaches, the oxygen saturation of the blood in the fœtal carotid artery falls to a level not much above that of the umbilical artery, so that the respiratory centre is now more easily released from inhibition.‡

With the first breath taken by the newborn child, all the mechanical conditions of the circulation are modified. The resistance to the blood flow through the lungs being diminished, the blood passes from the pulmonary arteries through the lungs into the left auricle. The pressure in the left auricle is thus raised, while that in the right auricle falls, so that the foramen ovale is maintained closed. Even before birth, proliferation of the lining membrane may be seen both in the ductus arteriosus and in the ductus venosus; and with the mechanical relief of the vessels afforded by respiration and the changed conditions of the fœtus, this proliferation goes on to complete obliteration of the vessels.

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VITAMINS IN RELATION TO REPRODUCTION

Animals in a poor state of nutrition do not reproduce readily, so that in conditions of vitamin lack some faults of reproduction are not unusual. Two of the vitamins, however, seem to be especially concerned, viz. vitamin E and vitamin A.

Vitamin E (*v.* p. 858) is essential in the diet of some of the lower animals, *e.g.* rats, if reproduction is to occur. Lack of it, although not apparently interfering with the normal reproductive processes of the female up to the eighth day of gestation, ultimately leads to retarded growth of the fœtus,

* COREY. *J. Exp. Zool.*, 1932, **61**, 1.

† HUGGETT. *J. Physiol.*, 1930, **69**, 144.

‡ BARCROFT, BARRON, COWIE and FORSHAM. *J Physiol.*, 1939.

failure of proper development of the vascular system and allantois, and ultimate death of the fœtus. The maternal placenta develops normally. The vitamin can apparently be stored in the body for several weeks, *i.e.* actual administration of it during pregnancy is not essential, provided that plenty has been given beforehand.

In the male rat, lack of vitamin E leads to atrophy of the seminiferous tubules.

Vitamin A also plays some part in the proper maturation of the gonads, since lack of it leads to abnormalities in the œstrus cycle, and delay in reaching maturity.

PARTURITION

In the first half of pregnancy the uterus is quiescent, and this is attributed to the inhibitory effect on it of progesterone. It is also unresponsive to oxytocin. Later, contractions of rhythmic character appear, at first feebly, then more powerfully. These contractions ordinarily give rise to no sensations, but are felt when they are augmented in consequence of reflex stimulation. During the greater part of pregnancy they have little or no effect on the contents of the uterus. During the last weeks or days of pregnancy, however, these contractions, which have now become more powerful, have a distinct physiological effect. Not only do they, by pressing on the fœtus, cause it, in most instances, to assume a suitable position for its subsequent expulsion, but, affecting the whole body of the uterus, including the longitudinal muscular fibres surrounding its neck, they assist the general enlargement of the organ in dilating the internal *os uteri*, so that the upper part of the cervix is obliterated and drawn up into the body of the uterus some little time before labour has commenced. Towards the end of term also, the uterus becomes sensitive to oxytocin, and this may be a fact of importance.

With these changes in the uterus are associated changes in the round ligaments and in the vagina and vulva. The muscular fibres of the round ligaments become much hypertrophied and lengthened, and these structures can therefore appreciably aid the uterine contractions in the subsequent expulsion of the fœtus. The vaginal walls become of looser texture, and so afford less resistance to distension during the passage of the fœtal head.

Considerable discussion has taken place as to the cause for the onset of labour or parturition, which in man occurs at a nearly constant period of 281 days after conception. It is perhaps significant that the period of gestation is a multiple of the sex cycle (œstrous in animals, menstrual in women). Most of the explanations which have been suggested, such as the great irritability of the uterus at the termination of pregnancy, the loosening of the fœtal membranes, the return of the menstrual congestion after ten missed periods, thrombosis of the placental sinuses, simply replace one question by another. Doubtless the reason why labour occurs at a definite time must be sought for in extrinsic, rather than in uterine, changes. It is probably hormonal in causation, and the problem may be viewed from an interesting angle if we inquire why it is that the presence of the fœtus is tolerated for so long. The quiescence of the uterus is now generally attributed to the production of progesterone ; if, in the rabbit, ovulation is artificially induced on the twenty-fifth day of pregnancy, by injection of a dose of the luteinising principle in human pregnancy urine, it is found that parturition does not occur at the normal time of thirty-two days, but at the fortieth day. This is associated with the presence of maximal-sized corpora lutea at the thirty-

second day, whereas in a normal pregnancy these are showing degeneration at that time.

The presence of the fœtus is not a necessary condition for the continuance of pregnancy, but the placenta appears to be important. If in pregnant mice the fœtuses are destroyed at the twelfth to fifteenth day of pregnancy, the placenta being retained, 'pseudo-parturition' with delivery of the placentæ takes place at normal full term,* and is followed, as is normal delivery, by an œstrus period. This indicates that the placenta plays an important part in inhibiting œstrus and so in determining the date of parturition: it also suggests that an œstrus-producing factor is operative at about that time. It is attractive to speculate that the sudden drop in the excretion of combined œstrin and the accompanying increase in the excretion of free œstrin which immediately precede delivery in the human subject may be decisive factors, since it has been shown that the injection of large doses of œstrin into pregnant animals at any stage leads to abortion.

A popular view at present is that a factor which plays an important part in the onset of parturition is the increasing sensitiveness of the uterus to the action of oxytocin, produced by the posterior pituitary, owing to a reduction in the inhibition exercised up to that time by the presence of progesterone. It is unquestionable that the administration of oxytocin causes rhythmic contractions of the parturient uterus very similar to those of normal labour (Burn and Bourne †), and it is also true that in some species abortion follows removal of the ovaries or of the corpora lutea. Removal of the pituitary, however, does not seem to affect the time of onset of parturition,‡ though there is evidence of difficulty in the deliveries. Moreover, although it has been shown that oxytocin produces greater uterine responses when œstrin is also present,§ this has not been substantiated by experiments on excised uteri treated with these substances ||

According to Moir, the uterine contraction is a peristaltic one, passing downwards towards the cervix, the contraction of which comes last, and is feeble. The cervix responds differently from the body of the uterus to drugs, and possibly has a different innervation. It has been shown by Newton¶ that the cornua of the pregnant goat's uterus respond to oxytocin whereas the cervix is insensitive, while adrenaline relaxes the excised cornua and contracts the cervix.

Actual parturition, in the woman, is generally divided into three stages. In the first stage the contractions therefore chiefly act in dilating the os uteri. In this dilatation two factors are involved, namely, the active dilatation brought about by the contraction of the longitudinal muscular fibres which form the chief constituent of the lower part of the uterine wall; and in the second place, a passive dilatation by the pressure of the fœtal bag of membranes, which is filled with amniotic fluid, and forced down as a fluid wedge into the os by the contractions of the uterine fundus. The uterine contractions are essentially rhythmical, being feeble at first, and increasing gradually in intensity to a maximum which endures a certain time, and then gradually subsides. The frequency and duration of the contractions increase as labour advances.

* NEWTON. *J. Physiol*, 1935, **85**, 196.
† BURN and BOURNE. *J. Obstet. and Gyn*, 1927, **34**, 249; *B. M. J.*, 1930 (ii), 87.
‡ DOTT. *Q. J. Exp. Phys*, 1923, **13**, 241; SMITH, *Am. J. Phys.*, 1932, **99**, 345; ALLAN and WILES, *J Physiol.*, 1932, **75**, 23.
§ MARRIAN and NEWTON. *J. Physiol.*, 1935, **84**, 133.
|| NEWTON. *J. Physiol.*, 1933, **79**, 301.
¶ NEWTON. *J. Physiol.*, 1934, **81**, 277.

The second stage begins as soon as the os uteri is fully dilated and the fœtal head has entered the pelvis, when the contractions change in character, being much more prolonged and frequent ('labour pains') and attended by more or less voluntary contractions of the abdominal muscles. This action of the abdominal muscles is associated with fixation of the diaphragm and closure of the glottis, so that pressure is brought to bear on the whole contents of the abdomen, including the uterus. No expelling force can be ascribed to the vagina, since it is too greatly stretched by the advancing fœtus. In this way, the fœtus is gradually thrust through the pelvic canal, dilating the soft parts which impede its progress, and is finally expelled through the vulva, the

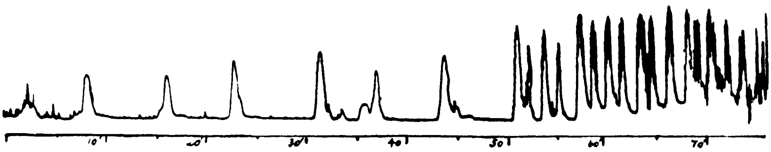

FIG. 666. Record of intra-uterine pressure of woman in labour. Note first stage pains up to the 50′ mark. Second stage pains recorded during next twenty-five minutes ; they begin suddenly. The head of the child was born at 75′ where the pressure momentarily drops, and the body just after the last rise of the lever. The patient was a multipara. (BOURNE and BURN.)

head normally being born first (Fig. 666). The membranes generally rupture towards the end of the first stage of parturition.

A third stage of labour is generally described. This consists in a renewal of uterine contractions about twenty to thirty minutes after the birth of the child, and results in the expulsion of the placenta and decidual membranes, and the closing, by compression, of the venous sinuses.

Nervous Mechanism. We possess little experimental knowledge of the nervous mechanism of parturition. Goltz observed the normal occurrence of heat, impregnation, and parturition in a bitch whose spinal cord had been completely divided in the dorsal region during the previous

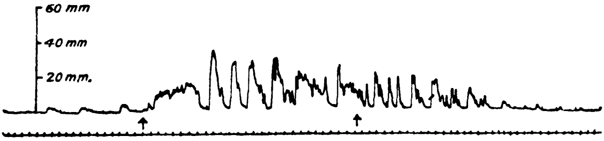

FIG. 667. Contractions of Human Puerperal Uterus. Suckling (between the arrows) caused increased contractions. (After MOIR. *Edin. Med. J.*, 1934, **41**, 93.)

year. On the other hand, destruction of the lumbo-sacral cord completely abolishes the normal uterine contractions of parturition, so that this act must be regarded as essentially reflex, presided over by a controlling 'centre' in the grey matter of the cord. The activity of the centre can be inhibited or augmented by impulses arriving at it from the peripheral parts of the body, as by the stimulation of sensory nerves, or from the brain, as under the influence of emotions. The nerve paths from the centre to the uterus have been already described.

THE PUERPERIUM. After delivery the uterus undergoes involution and lactation soon commences. In the latter phenomenon the lactogenic hormone of the pituitary plays a decisive *rôle.* Suckling of the child is important in accelerating the involution of the uterus, which is caused to enter into powerful rhythmic contractions each time the child is put to the breast (Fig. 667). Excretion of œstrin and of gonadotropic hormones in the urine

rapidly falls off and reaches the normal level in a week or ten days. Menstruation recommences when lactation is finished.

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CHAPTER LIII

THE SECRETION AND PROPERTIES OF MILK

LACTATION

AFTER birth the quality of the nutriment supplied to the child normally depends on the supply of milk by the mammary glands. Nutrition now involves further activity on the part of the infant, the alimentary canal being concerned in the digestion of the milk supplied by the mother, and the kidneys being made use of for getting rid of waste material.

Growth and Functional Differentiation of the Mammary Glands. The preparation of the mammary glands for the subsequent nourishment of the newborn child begins in the first month of pregnancy, and is marked by swelling of the glands, rapid proliferation of the duct epithelium, and production of many new secreting alveoli. As we have seen, there are probably at least three hormonal factors concerned in these processes—œstrogen and progesterone from ovaries and placenta, and prolactin from the anterior pituitary. The œstrogens cause growth, chiefly of the duct system, and of the epithelium of the nipple, and progesterone causes growth and differentiation, up to the threshold of lactation, in the secreting alveoli. Œstrogens and progesterone are, however, ineffective in causing mammary growth in hypophysectomised animals, though the effect on the nipple is obtained. Implantation of pituitary restores the proliferating effect, and hence collaboration between a pituitary hormone, œstrogen and progesterone seem essential at an early stage, as well as later. These same hormones are also available in the course of the normal menstrual cycle, and are no doubt responsible for the periodical enlargement of the breasts at each menstrual period. Throughout these changes, and throughout pregnancy, however, the secretory activity of the enlarged glands is held in abeyance. With the expulsion of the fœtus at birth and the breakdown of the corpus luteum, the source of the inhibitory stimulus is removed, the overgrown gland cells enter into a condition of activity, and the secretion of the gland, once formed, is continued independently of the fœtus, or, indeed, of any of the pelvic organs. Removal of the ovaries in a cow is, in fact, sometimes employed as a means of prolonging the secretion of milk. For this initiation and maintenance of secretion, the lactogenic hormone of the anterior pituitary (prolactin) is mainly responsible. Certainly lactation is at once arrested after hypophysectomy,* while on the other hand, if preliminary breast growth be induced, by previous dosage with œstrogen and progesterone, injection of the lactogenic hormone will cause lactation in immature females, or even in males. It has been successfully employed clinically in cases of failure of lactation.† Prolactin can be assayed by the determination of the growth which it causes in the crop glands of pigeons or doves of either sex, as compared with that caused by an international standard of which the unit is 0·1 mg.‡

* SELYE, COLLIP and THOMPSON. *Endocrinol.*, 1934, **18**, 237.
† RIDDLE. *J. Amer. Med. Assoc.*, 1935, **104**, 636.
‡ *Bull. Health Org. League of Nations*, 1939, **8**, 909.

During the latter half of pregnancy, a watery fluid can generally be expressed from the nipple. In certain mammals, this watery secretion gives place to a secretion of true milk at the end of gestation, or during the process of parturition itself. In the woman, though the formation of milk may be hastened if a child has been put to the breasts during the latter part of pregnancy, secretion normally begins on the second or third day, even if the child has been born dead and no attempt at suckling has taken place. Normally, the only condition which is necessary for secretion to continue during six to nine months after parturition is the repeated removal of the secreted milk. The process of suckling not only removes the milk already secreted but excites the secretion of more milk. This it probably does by causing reflexly an increased formation of lactogenic hormone in the anterior pituitary.* The reflex may originate from the nipples, but the release of tension on the uterine walls seems to be an essential condition, since emptying of the uterine contents may precipitate lactation, while distending the

TABLE SHOWING AMOUNT OF MILK SECRETED BY A NURSING WOMAN

INCREASE

Time	Milk secreted daily
1st day	20 grammes.
2nd ,,	97 ,,
3rd ,,	211 ,,
4th ,,	326 ,,
5th ,,	364 ,,
6th ,,	402 ,,
7th ,,	478 ,,
2nd week	502 ,,
3rd–4th week	572 ,,
5th–8th ,,	736 ,,
9th–12th ,,	797 ,,
13th–16th ,,	836 ,,
17th–20th ,,	867 ,,
21st–24th ,,	944 ,,
25th–28th ,,	963 ,,

DECREASE

Time	Milk secreted daily
29th–32nd week	916 grammes.
33rd–36th ,,	909 ,,
37th week	885 ,,

emptied uterus with paraffin wax delays or inhibits it. An increase of blood sugar, or administration of thyroxin, cause in cows an increase in the volume and concentration of the milk.† If the woman does not nurse her child, the swelling of the breasts gradually passes off, the milk disappears, and the glands undergo a process of involution. Under normal conditions the secretion of milk lasts for six to nine months, and may in rare cases extend over more than a year. The amount secreted increases at first with the growth and size of the child, and often may reach 1·5 to 2 l., or occasionally 3 l. daily. The Table above represents the average amount of milk secreted during the thirty-seven weeks after birth. It will of course be greater with strong, big children, and smaller with weakly children.

COLOSTRUM. Before the secretion of true milk begins, the fluid which is obtained from the breasts is known as colostrum. It may be expressed from the breasts immediately after birth and is ingested by the child during the first two days after birth. The colostrum is formed only in slight quantities. It is an opalescent fluid,

* SELYE. *Amer. J. Physiol.*, 1934, **107**, 535.

† FOLLEY and WHITE. *Proc. Roy. Soc.*, 1936, **120 B**, 346.

often somewhat yellowish, containing fat globules which, if the fluid be allowed to stand, form a yellowish layer on the top. Under the microscope, in addition to the fat globules, may be seen the so-called colostrum corpuscles, which are multinucleated cells loaded with particles of fat. They are probably leucocytes or phagocytes which have wandered into the alveoli and have taken up fat globules. Some of the corpuscles may be desquamated secretory cells. Colostrum is distinguished from true milk by containing little or no caseinogen. It contains about 8 per cent. of proteins, namely, lactalbumin and lactoglobulin, which coagulate on boiling. Lactose and fat are present in smaller, and salts in greater amount than in ordinary milk. It is popularly supposed to have a laxative effect on the child.

PROPERTIES OF MILK

Fully formed milk presents certain features which are common to all mammals. We may therefore deal with the composition of cow's milk and point out later in what respect human milk differs therefrom. Milk is an opaque, white fluid, with characteristic odour and sweetish taste. Its specific gravity varies between 1028 and 1034. The $pH = 6{\cdot}6$—$6{\cdot}8$, and, as it contains buffers, *e.g.* phosphates and caseinogen, it may be titrated, with suitable indicators, against either acids or alkalies. One hundred cubic centimetres of average fresh milk, when titrated to methyl orange, require 41 c.c. $n/10$ acid for neutralisation. When titrated to phenolphthalein the same amount requires 18 c.c. $n/10$ alkali for neutralisation. When exposed to the air, milk rapidly undergoes changes in consequence of infection by micro-organisms. The most common of these changes is the formation of lactic acid by the *bacillus lacticus*. In some cases the milk may undergo a species of alcoholic fermentation, as in the formation of *kephir*, which is made by the fermentation of mare's milk.

Composition of Milk. FATS. The opaque appearance of milk is due chiefly to the presence of multitudes of fine fatty particles. On allowing the milk to stand, or by centrifugation, the particles rise to the surface, forming cream, and by mechanical agitation, especially if the milk is slightly sour, they may be caused to run together with the formation of butter. The reason why the fat globules do not run together naturally is probably because, in any protein solution, such as that in which the globules are suspended, the protein tends to aggregate, with the formation of a pellicle, at the surface, so that an emulsion once produced in such a fluid will tend to be more or less permanent. The fats of milk consist for the greater part of the neutral glycerides, tripalmitin, tristearin, and triolein. In smaller quantities it contains the triglycerides of butyric acid, and all other saturated fatty acids of even carbon numbers up to lignoceric (C_{24}), and various unsaturated acids also. It also contains some lecithin and cholesterol.

The milk plasma, the fluid in which the fat globules are suspended, contains various proteins, lactose, and inorganic salts, with a small amount of lecithin and nitrogenous extractives.

PROTEINS. The chief protein of cow's milk is *caseinogen*, a phosphoprotein soluble in dilute alkalies. It may be prepared from separated milk by the addition of weak acids. The precipitate may be washed by decantation, and purified by solution in dilute ammonia and precipitation by acetic acid two or three times. The precipitate is extracted with alcohol and ether, and forms a snow-white powder which is practically insoluble in water and dilute salt solutions. It is easily dissolved on the addition of a little alkali, when it yields solutions which are acid to litmus. When rubbed up with chalk it dissolves, displacing carbon dioxide and forming a calcium caseinogenate. A solution of caseinogen in soda or potash is transparent and passes easily through a clay cell. The calcium caseinogenate forms opalescent

solutions. Apparently the compound is dissociated by water with the formation of caseinogen acid which is in a state of partial solution as swollen-up aggregates. It is impossible, therefore, to filter calcium caseinogenate through a clay cell. It is mainly in this form that caseinogen is contained in milk, hence the opalescent appearance of the milk plasma. On treating caseinogen with rennin, it is converted into a modification known as *paracasein*, which in the presence of calcium salts is thrown out as insoluble *casein*. To this process is due the clotting of whole milk by rennin, which is made use of in the preparation of cheese, the curd consisting of a network of casein enclosing fat globules in its meshes. On allowing the clot to stand it shrinks, pressing out the whey.

From the whey may be obtained two other proteins, known as *lactalbumin* and *lactoglobulin*. These resemble the albumin and globulin of blood serum. They are coagulated on heating, and form a pellicle on the surface, as milk does. In human milk there is more lactalbumin (about 0·7 per cent.) than caseinogen (about 0·3 per cent.). According to some authors a third protein is present in the whey, to which the name whey protein has been given, and which is supposed to be split off from the caseinogen under the action of rennin.

Milk can be boiled without undergoing general coagulation. If it be allowed to stand and become sour by the formation of lactic acid, at a certain degree of acidity boiling the milk causes its complete coagulation. Later on the acid produced is sufficient in itself to precipitate the caseinogen. Both these processes—namely, coagulation of half-sour milk by heating, and spontaneous clotting of milk by the production of acid—are made use of in different countries for the manufacture of cheese.

Carbohydrate. The lactose is most easily obtained from whey, which is boiled to precipitate the remaining proteins. On filtering and evaporating slowly, the milk sugar crystallises out. Lactose is a disaccharide which on hydrolysis by acids, or by the lactase of the intestinal juice, yields glucose and galactose. It may be found in the urine of nursing women when the breasts are not kept empty, owing to reabsorption of the lactose formed in the mammary glands.

Salts. The salts of milk include calcium, sodium and potassium as phosphates, citrates and chlorides. It is said to be deficient in iron.

Significance of Constituents. The composition of milk depends on many factors, such as diet, health, frequency of milking, exercise, emotional states, etc. Human milk shows a change in composition in the course of lactation, as shown in the Table below, the protein declining, the lactose and fat increasing slightly.

Average Composition of Human Milk (After Bell *)

Time.	Protein.	Lactose.	Fat
5 days	2·00	6·42	3·2
9 „	1·73	6·73	3·7
3–4 weeks	1·37	7·11	3·6
5–6 „	1·30	7·11	4·0
7–8 „	1·21	7·11	4·0

Milk contains vitamins A, B_1, B_2, C and D in adequate amount.

* Bell. *J. Biol. Chem.*, 1928, **80**, 239.

Milk is excellently adapted to the needs of the growing animal. In the first place, we find a proportionality between the total solids of the milk and the rate at which the young animal grows. The milk taken by the animal serves only in part for the production of energy, a great proportion of it being required for the building up of new tissue. In no respect is this correspondence seen better than in the comparative analyses of the ash of milk and of the young animal of the same species. Thus, the calcium content of human milk is 0·04 per cent., and that of rabbit's milk about 0·9 per cent.

This close correspondence is necessary only where growth is very rapid, so that the greater part of the constituents of the milk have to be utilised in the building up of the animal tissues. The slower the growth of the animal the greater the divergence between the composition of the milk and that of the newborn animal.

The relation between rate of growth and protein content of the milk is well illustrated by a comparison of the composition of the milk in different animals. In the following Table (Proscher) it will be seen that the more rapidly an animal grows the greater is the protein content of the milk with which it is supplied :

	Time in which the body weight of the newborn animal was doubled Days.	Protein Per cent
Man	180	1 6
Horse	60	2 0
Cow	47	3 5
Goat	19	4 3
Pig	18	5 9
Sheep	10	6 5
Dog	8	7 1
Cat	7	9 5

We should expect that the milk, which is the sole food of the growing infant, would contain a relatively greater proportion of protein than is necessary for the adult. In an experiment by E. Feer, quoted by Bunge, a child weighing 8226 grammes at the thirtieth week took 951 grammes of milk. Human milk contains :

Protein	1·6 per cent.
Fat	3·4 ,,
Sugar	6·1 ,,
Ash	0 2 ,,

The child was therefore receiving daily :

Protein	15·4 grammes
Fat	32·3 ,,
Sugar	58 0 ,,
Ash	1·9 ,,

According to the same proportions a man of 70 kilos. fed entirely on milk would take in

Protein	129 grammes.
Fat	275 ,,
Sugar	494 ,,
Ash	16 ,,

It is interesting to note that the protein of this diet differs but little from that in the diets ordinarily accepted as standard, but there is a large excess in the fat and in

the total Calorie value, as would be expected from the more rapid metabolism and the relatively larger body surface of the young child.

The fitness of caseinogen for building up the tissues of the body is evident when we compare the products of its hydrolysis with those of the proteins in other foodstuffs. It will be seen from the Table on p. 50 that practically every amino-acid and allied substance employed in the building up of the various proteins is represented in caseinogen. The only exceptions are glycine, which can easily be formed from other amino-acids, and cysteine, which is present, however, in lactalbumin.

We thus see that, under normal conditions, the young animal is supplied, through its natural food, with all the foodstuffs in the proportions which it requires for its normal nourishment and growth. It is impossible, therefore, satisfactorily to replace the natural milk of an animal by that of another species. In civilised communities it is becoming more and more the custom to endeavour to feed infants with cow's milk, more or less modified, in the vain endeavour to reproduce the properties of human milk. Among all classes, this involves the administering of a milk differing in its qualities and in the relative proportions of its proteins, fats, carbohydrates, and salts, from human milk. So-called ' humanised ' milk is only a rough imitation of the natural mother's milk. Among the poorer classes this artificial feeding means the replacement of a natural sterile food, throwing very little work on the digestive organs of the child, by a foreign milk, very difficult to digest and often teeming with micro-organisms. In some cases it is necessary to adopt artificial feeding because there is an insufficient secretion of milk. It is therefore important to know what are the main differences in composition between human and cow's milk. In human milk, the caseinogen is, not only absolutely, but also relatively, less than in cow's milk, while the latter is relatively poorer in lactose. Human milk is poorer in salts, especially in lime, containing only one-sixth of the amount present in cow's milk. The main differences may be summarised as follows :—

	Water	Proteins		Fat	Lactose	Salts
		Caseinogen	Albumin			
Human milk . .	88·5	0·3	0·7	3·7	6·7	0·2
Cow's milk . .	87·1	2·3	0·7	4·0	4·8	0·7

THE SECRETION OF MILK. When fully formed, each mammary gland consists of fifteen to twenty lobes embedded in connective tissue. Each lobe is made up of a mass of secreting alveoli, which lead by narrow ducts into one large lactiferous duct. These lactiferous ducts, one from each lobe, open on the nipple, undergoing in the nipple itself an oval enlargement. Before pregnancy, the alveoli as well as the ducts are lined with a cubical epithelium. Early in pregnancy a marked difference develops between the epithelium of the alveoli and that of the ducts.

The secreting cells increase in size and especially in height, and numerous small fat cells, mitochondria and spherical "pseudo-yolk" granules are present.* At later stages the fat globules increase in size and collect towards the lumen, while the pseudo-yolk granules disappear. When lactation sets in the cells contain elongated mitochondria and one or two large fat globules next to the lumen; ultimately the fat globules are

* KATHARINE R. JEFFERS. *Amer. J. Anat.*, 1935, **56**, 257.

discharged, together with a little cytoplasm, by a budding-off process, into the secretion in the lumen (Fig. 668). During the stage of enlargement and also during lactation there are frequent mitotic divisions in the alveolar cells. A few cells degenerate and are shed, as also are a certain number of leucocytes.

The secreting mammary gland is very vascular, about 300 to 400 volumes of blood being needed for each volume of milk produced.

We know very little about the mechanism of milk secretion. It seems impossible at present to explain the very close adaptation between the activity of the secretory cells and the needs of the infant or young animal. Two at least of the constituents of milk, caseinogen and lactose, are peculiar to this secretion. It would seem that the caseinogen is produced by the gland cells by synthesis from the amino acids of the blood stream, and that

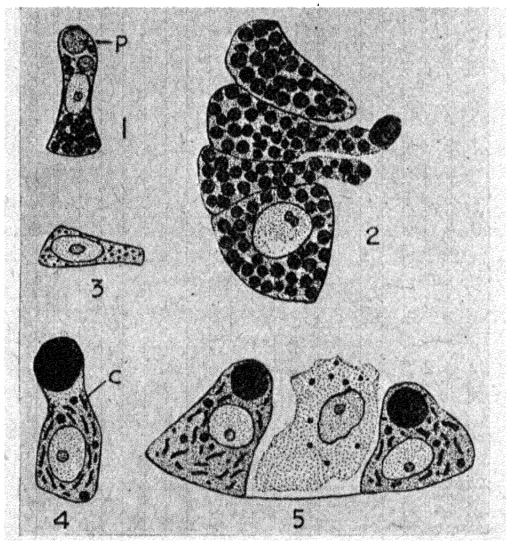

FIG. 668. Cells of Mammary Gland of Rat. (1) and (2) On eighteenth day of pregnancy: fig. 2 shows elimination of a large fat drop. (3) A cell after fat has been secreted, a few hours before parturition. (4) Cell in full lactation, seven days after parturition. (5) Two normal cells and one that is degenerating and passing into the lumen; twelfth day of lactation p = pseudo-yolk spheres, c = chondriosomes. (JEFFERS. *Amer. J. Anat.*, 1935, **56**, 257.)

the lactose is derived, also by synthesis, from the blood sugar. Slices of surviving lactating mammary gland can form lactose from glucose, but not from fructose, mannose or galactose.* The fat similarly originates in the blood fat,† and much of it ultimately in the fat of the food, or of the body depots. The phosphorus compounds of milk are derived from the inorganic phosphate of the blood.

The food of the mother may be varied within very wide limits without altering the composition or amount of the milk secreted, provided that the food is sufficient in amount. The only constituent of the milk for which we have direct evidence of alteration by changes in the food supply of the mother is the fat. It is well known that the composition of butter may be affected according to the food supplied to the cow. A large supply of oilcake,

* GRANT *Bioch. J.*, 1935, **29**, 1905; 1936, **30**, 2027

† GRAHAM and KAY. *Proc. Roy. Soc.*, 1936. In Press.

for instance, may result in the production of a butter which is deficient in the saturated fatty acids and is therefore oil at ordinary temperatures. Abnormal fats and fatty acids such as iodised fats or erucic acid, when administered to an animal in lactation, may appear among the fats of the milk. Not only can the secretion and composition of the milk be affected reflexly through the nervous system, as *e.g.* under the influence of emotions, but the influence may be reciprocal. This is especially marked in the case of the pelvic organs. The act of suckling excites rhythmic contractions of the uterus (*v.* Fig. 667). Putting the child to the breast shortly after birth is, therefore, an important means of causing contraction of the uterus, and stopping any tendency to hæmorrhage from the venous sinuses opened by the separation of the placenta and fœtal membranes. The nursing of the child is thus an important means of procuring a proper involution of the uterus after labour.

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CHAPTER LIV

HEREDITY

From the fertilised egg, there is formed an individual partaking of many of the minutest characteristics of both its parents. An egg cell has much the same appearance, whether it belong to an echinoderm, a fish, or a woman. In the process of development, by a simple repetition of a series of cell divisions, this undifferentiated protoplasm is formed into the complex organs with the potentialities and habits which distinguish the type from which it has been derived. There must, therefore, be some difference between different eggs. We cannot wonder that the intimate nature of this process has been the subject of speculation from the very birth of science. Running through these speculations were two main ideas, which have been labelled the theories of 'evolution' and of 'epigenesis.' By the 'evolutionists' the egg was believed to contain an embryo fully formed in miniature, as the bud contains the flower or the chrysalis the butterfly. Development, therefore, was only the unfolding of something already existing. If, however, this theory be pushed to its utmost and if the egg contain a complete embryo, this must itself contain eggs for the next generation, and so on *ad infinitum*, a conclusion which of course is absurd. According to the theory of epigenesis, the structure of the egg is wholly different from that of the adult, its development into the new individual consisting in the continual formation, one after the other, of new parts previously non-existent as such. There is no doubt that this view is fundamentally correct. The difficulty with which we have to contend is the understanding of the orderly sequence and correlation of the cell divisions and differentiations which result in an adult individual of the same type as the parents. The fact that, under approximately identical conditions, one mammalian ovum will give rise to a mouse and the other to a man, indicates that there must be some preformed differentiation in the nuclear structures, *i.e.* chromosomes, of the ovum itself. We have already seen that in some types the germ cells are separated off from the rest of the embryo at a fairly early stage. If, in the two-celled stage of the frog's egg, one cell be destroyed by means of a hot wire, the other cell develops to form half an embryo, thus suggesting that each cell of the two-celled embryo could give rise only to the corresponding half of the body. This limitation of development, however, occurs only if the intact cell be left in connection with the cell that has been injured. If, in echinoderm larvæ, the cells be entirely separated, even as late as the eight-celled stage of division, each cell will give rise to a whole embryo, differing from a normal embryo only in respect of size. In man too, 'identical twins' result similarly, from separation of the two cells formed by the first division of the fertilised ovum. This suggests that the number of divisions that each cell can undergo is predetermined in the egg cell itself, but shows also that the cells into which the egg divides are, at first at any rate, equipotential. We must assume, therefore, that the reason why one cell under ordinary circumstances forms only one half of the embryo is that its development is regulated and determined by the presence of the other cell in connection with it and forming the other half of the embryo. That is to say,

the development of the egg involves the adaptation to environment of a protoplasm of certain properties and powers of reaction. The final product of development depends (1) on the nature of the protoplasm (including the nucleus) of which the egg is composed, and (2) on the environmental conditions to which the egg is subjected during the rapid growth and multiplication attending its development. We could therefore speak of a morphology of inheritance, but the morphology would be ultra-microscopic and have relation to the molecular structure of the protoplasm of which the egg was composed.

In the transmission of the potentialities of development, from parent to fertilised egg, we must regard the nucleus as the essential structure. In ordinary development the spermatozoon furnishes only half a nucleus and a centrosome, the ovum supplying the other half nucleus, and the whole of the cytoplasm. In division of the egg, the only part of the cell which can divide so that each daughter cell shall include an equal part of both parental germs is the nucleus. The constant number of the chromosomes in each species, and their accurate division on mitosis, suggest that the hereditary transmission of the potentialities of the cell is bound up with the chromosomes. It has been suggested that every inheritable character is located in a chromosome, or part of a chromosome. If this be the case, one might regard the differentiation into various tissues, which occurs in the process of development, as occasioned by an actual loss or degeneration of the constituent parts of one or more of the chromosomes present in the pluripotential fertilised egg-cell. There is no doubt that many tissues do become thus differentiated at a fairly early period in development, having undergone in the process an absolute modification of their potentialities, which must be at any rate shared by the chromosomes of their constituent cells. The extent to which this limitation of powers of development occurs varies widely in different animals. In the higher animals, such as man, epithelium will reproduce epithelium and liver cells will reproduce liver cells, while nerve cells once definitely characterised are absolutely incapable of multiplication. On the other hand, in crustacea, a whole limb may be torn off and be regenerated from the tissues of the stump. Destruction of the optic lens in the salamander is followed by its regeneration from the anterior part of the optic cup, a tissue which had no part in its primary formation. Worms will form a new head after decapitation. In these animals, therefore, the cells in many parts of the body possess the power of directed growth, if need arise in case of injury, and are able to form tissues of many different kinds.

We have mentioned the small size of the larvæ formed from isolated cells of the segmenting egg as a proof that the number of cell divisions of the somatic part of the developing animal is predetermined and limited. This conclusion must not be taken too absolutely. Many of the tissues, even of the highest animals, possess the power of almost unlimited regeneration by cell multiplication as a response to injury. Under normal conditions, the growth of such tissues is limited, not by absence of power to divide, but as a result of a mutual interaction between them and the surrounding cells. We might almost speak of a struggle for existence between the various tissues of the body, which, in the healthy organism, results in a balance of multiplicative powers. If this balance is upset by any means, such as stimulation of certain cells by the presence of intracellular parasites, or their destruction by irritants or other abnormal conditions (*e.g.* exposure to X-rays), one tissue may enter into active growth at the expense of the surrounding tissues, and the result is a morbid growth such as cancer. It is possible that in the latter case a new factor comes into play. All tissues

of the body, as we have seen, begin to die from the time that they are born. They have a certain span of life, a certain limitation to their generations, which results in the phenomenon of senescence, such as occurs in a culture of protozoa. In protozoa this phenomenon is the signal for rejuvenation by conjugation. It is possible that, in cancer, something of the same nature occurs. It is, at any rate, significant that in a rapidly growing cancer, many of the dividing cells present the phenomenon of meiosis, a phenomenon which is otherwise found only in the ripening sexual cells. Given adequate conditions of nutrition, there seems to be no limit to the growth of cancer cells. In mice, a cancer may be transferred from one individual to another by inoculation, and this process may apparently go on indefinitely.

In sexual reproduction, the new individual partakes of characteristics of both its parents. It therefore resembles neither of its parents in all details. The conjugation of the two parent cells, from which it is derived, has been preceded by a throwing out of half the chromosomes from each parent cell. It is therefore natural to ascribe the variations, which occur among the members of one family, to a qualitative difference in the chromosomes which have been eliminated in the formation of their respective egg cells. Can we regard the chromosomes as representing separate qualities of the individual, or must we assume that all qualities are represented to a greater or less extent in every chromosome ? In the case of many qualities, especially those which distinguish the species as apart from the individual variation or family characteristic, we must probably accept the latter idea as correct. In this case, the child can be regarded as representing an arithmetical mean of both its parents. In certain respects, however, a quality seems to be transmitted from parent to offspring, either completely or not at all. This is specially applicable to those characters which have been rapidly produced by artificial selection, characters which, if artificial selection be abandoned, rapidly disappear, with reversion to the type from which the special strain was initially produced. These characters are alternative or *allelomorphic*, *e.g.* tallness or shortness, blue eyes or brown eyes.

LAWS OF INHERITANCE

Mendel's First Law. The way in which these characteristics are transmitted was first studied by Mendel and has been formulated as 'Mendel's laws.' *The first law* is that of the *segregation of characters*, and is illustrated by his first experiments, which were carried out on peas. On crossing a tall plant with a dwarf plant, seeds were obtained all the plants from which were tall. On recrossing the plants of this generation among one another, a third generation (F_2) was obtained, in which 25 per cent. of the plants were dwarfs and 75 per cent. were tall. Crossing the dwarf plants among themselves led to the production of dwarf plants through successive generations. Of the 75 per cent. tall plants one-third, and all their descendants, continued to produce tall plants when self-fertilised, whereas of the remaining two-thirds of the tall plants 25 per cent. produced dwarfs and 75 per cent. produced tall plants. On continuing the process of breeding, the dwarf plants, when self-fertilised, always produced dwarfs, whereas of the tall plants 25 per cent. produced tall plants which bred true, while the remaining 50 per cent. produced the same percentage of tall and dwarf as in the preceding generations. Mendel explained these results by the assumption that a character could be *dominant* or *recessive*. If both characters were present together in one plant it would partake of the dominant type; the fact that this plant possessed the recessive character would be shown only by the result of breeding. In the case of the peas, the tall character was dominant over the dwarf. Thus when the tall and dwarf pea were crossed, the first generation of plants would exhibit the dominant character and be tall. In the second generation, however, 25 per cent. of the individuals would be pure dominants (D + D), 25 per cent. would be pure recessives (R + R), while 50 per cent. would be mixed (D + R). The pure domi-

nants bred together would always give rise to nothing but pure dominants, the recessive to recessive, while the mixed type would always, as before, give rise to 25 per cent. pure dominants, 50 per cent. mixed, and 25 per cent. pure recessives. These results may perhaps be made clearer by the Table below :—

```
                             D + R
                               |
                               DR
                               |
      ---------------------------------------------------
      |                        |                        |
  25% D                    50% DR                   25% R
      |                        |                        |
      |       |          ------------          |        |
      D   25% D            50% DR         25% R        R
      |       |                |               |        |
      |       |      |   ------------   |      |        |
      D       D  25% D     50% DR     25% R    R        R
```

It has been suggested that a very large number, if not all, of the characters of an individual might be brought under this law. This might be done by indefinitely subdividing the characters, but the question would then become beyond the limits of analysis or experimental investigation. There is no doubt that many qualities are subject to Mendel's law, and their study has been of considerable assistance in guiding the efforts of our breeders and horticulturists in the formation of new varieties desirable for their value to man.

In respect of many qualities, the Mendelian idea of dominance seems to fail. Thus, in man, the progeny of a cross between a white and a black race are more or less intermediate between the two, and vary according to the amount of black and white blood introduced in succeeding generations. Definite black and white individuals are not produced, but merely individuals of various degrees of brownness. This is known as *blending.*

The explanation now given of Mendel's first law is a simple one. The inheritable factors or *genes* are located in the chromosomes, and these are paired in the somatic cells, but single in the gametes. When the two factors of an allelomorphic pair are identical in the somatic cells, then the single factors in each gamete are identical, and the condition is called *homozygous* , if they are different, it is called *heterozygous*, in which case half the gametes will have one of the allelomorphic factors and the other half the other one. Suppose we take two characters T and t, representing tall and short respectively, and mate together a gamete from a pure tall plant and one from a short plant. The somatic cells of these are TT and tt and the gametes T and t respectively. The fertilised cell will be Tt and the new plant from this will produce gametes, half of which, owing to their formation by reduction division, will be T and half t. If these are mated together, then by the law of chance we should get the combinations TT, Tt and tt in the ratios 1 : 2 : 1 (3 dominants to 1 recessive), and, of these, the first and last being homozygous would breed true, while the second being heterozygous would, on subsequent propagation, segregate in a similar way into pure and mixed types.

Mendel's second law relates to the *independent association of genes*, and states that the various possible allelomorphic pairs are independent of each other in inheritance. For instance, if we consider two factors Aa and Bb, in which A and B are dominant ; and cross, say, an individual who is BBaa with one who is bbAA, then the gametes of the first will be Ba and of the second bA, and the somatic cells resulting will be Bb Aa. These will produce gametes of types BA, Ba, bA and ba, in equal numbers, and when they are crossed, in accordance with the laws of chance, the offspring will be of the types **BBAA, BBAa, BbAA, BbAa,** BBaa, Bbaa, bbAA, bbAa, and bbaa, in the ratios 1 : 2 : 2 : 4 : 1 : 2 : 1 : 2 : 1 or, in every sixteen :

9 with both dominants A and B.
3 with one dominant (A) and one recessive (b).
3 with one dominant (B) and one recessive (a).
1 with both recessive characters (a and b).

The 9 : 3 : 3 : 1 is obtained obviously by the combination of two 3 : 1 ratios of dominant to recessive characters, *e.g.* in peas, A = tall, a = short, B = purple flowers,

b = white flowers. If we cross a tall purple with a short white, the result will be all tall purples, but if these are self-pollinated we get :—

$$3:1\left\{\begin{array}{l}12 \text{ Tall } = \underbrace{9 \text{ purple} + 3 \text{ white},}\\ 4 \text{ Short} = 3 \text{ purple} + 1 \text{ white},\end{array}\right.$$

$$3:1,$$

or, rearranged :—

$$3:1\left\{\begin{array}{l}12 \text{ Purple} = 9 \text{ tall} + 3 \text{ short},\\ 4 \text{ White} = 3 \text{ tall} + 1 \text{ short},\end{array}\right.$$

$$3:1.$$

By similar independence, where three allelomorphic characters are considered, we get various combinations of dominant and recessive, in accordance with the laws of

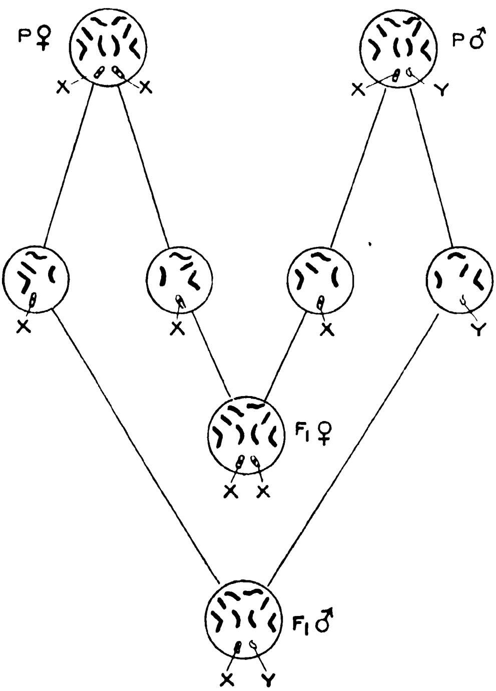

FIG. 669. Diagram to illustrate Principle of Sex-determination in an animal with 10 Somatic Chromosomes (5 pairs). P ♀, Female somatic cell. P ♂, Male somatic cell. G, The two ripe ova and two ripe spermatozoa (one X and one Y). F_1 ♀, Fertilised ovum which forms a female. F_1 ♂, Fertilised ovum which forms a male.

chance, with ratios 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1. The possible variations in crossings where an indefinite number of characters are considered is thus limitless.

The Determination of Sex

In many animals it is known, and in all it is probable, that one of the somatic chromosome pairs are different in the two sexes, one sex having a certain pair of chromosomes, the 'sex chromosomes,' which are represented by only one, or by one complete and one vestigial chromosome in the other sex, and there is good reason to suppose that the initial determination of sex is connected with this difference.*

When the reduction of the chromosomes takes place during gametogenesis and the individuals of each pair of chromosomes pass into different germ cells one complete sex

* McCLUNG. *Biol. Bull.*, 1902, **3**, 43 WINIWARTER. *Arch de Biol*, 1938, **49**, 119.

chromosome passes into each germ cell, in the sex that has two similar chromosomes, called XX (female in mammals); in the other sex where there is only one chromosome similar to these and the other one is either rudimentary (XY type) or lacking (XO), then one of the gametes will receive the X-chromosome, and the other one either no corresponding chromosome, or else the modified Y-chromosome. Thus the sex which is of XY or XO type (male in mammals) produces gametes of two kinds, while the gametes of the sex with the complete pair of sex chromosomes are of one type only. Thus when fertilisation takes place the original dimorphism of the zygotes is maintained. The chromosome mechanism at fertilisation is shown diagrammatically in Fig. 669.

The sex which produces dimorphic gametes is therefore 'heterozygous' for sex, while the other is 'homozygous.' The study of the correlation between sex and chromosome constitution has been greatly facilitated by the study of sex-linked characters, such as hæmophilia, their mode of inheritance lending strong support to the supposition that sex is initially dependent on chromosome constitution.

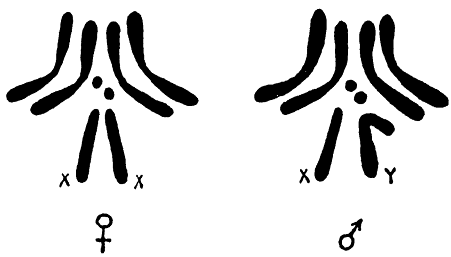

FIG. 670. Female and Male Groups of Chromosomes (Diploid) of *Drosophila melanogaster*. (After MORGAN, in Cowdray's *General Cytology*)

The sex carrying the extra chromosome varies in different groups of animals. In Lepidoptera and birds, for instance, the female is heterozygous and produces two kinds of ova, while the spermatozoa are of the same type, and in this case, therefore, the sex of the future embryo depends on the type of ovum fertilised. In Diptera and mammals (including man), on the other hand, the ova are all of the same type, but there are two kinds of spermatozoa. In the now well-known case of the fruit fly, Drosophila, there are four pairs of chromosomes, comprising three pairs of ordinary chromosomes in both sexes, together with an XX pair in the female, and an X and Y in the male (Fig. 670). The spermatozoa are, therefore, of two types, having three ordinary chromosomes and an X, or three ordinary chromosomes and a Y. Ova fertilised by the former produce females, and those fertilised by the latter produce males. In the human subject the male again is heterozygous, the somatic number of chromosomes (according to Painter, Evans and Swezy, and others) being 46 + XX in the female and 46 + XY in the male. The Y chromosome is the smallest. The spermatozoa, therefore, contain 23 + X or 23 + Y, so that fertilisation of ova (23 + X) by the former results in females, and by the latter, in males. There is little doubt that, normally, the modelling of the embryo to the male or female pattern is primarily dependent upon its chromosome constitution, but there is equally little doubt that, in rare cases, other factors may lead to the chromosome constitution being over-ridden, causing the embryo to be partially or entirely diverted to the sex to which it was not predestined, and resulting in the production of intersexes or sex reversals.

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Linkage

Mendel's second law is not of universal application, owing to the existence of what is termed linkage. Experiment shows that certain factors are inseparable or only partly separable from certain others. Some factors are sex-linked, a good example being in *Drosophila*. When a white-eyed male is mated to a wild red-eyed female, all the first generations (F_1) are red-eyed. When these are inbred, the F_2 consist of three red eyes

to one white, all the latter being males. Consideration of these results indicates that the eye-colour factor is transmitted by the X-chromosome but not by the Y. Representing (WX) as the chromosome with the linked gene, red + sex, and (wX) as = white + sex, we have in this case :—

P_1. (wX)Y × (WX)(WX)
(white-eyed male) (red-eyed female)
F_1 = (WX)Y : (WX)(wX) (all red-eyed)
(male) (female)
F_2 = (WX) WX) : (WX)(wX). 50 per cent. females; all red-eyed.
and (WX)Y, 25 per cent. red-eyed males,
and (wX)Y, 25 per cent. white-eyed males.

When, as a fresh experiment, a red-eyed male is mated with a white-eyed female, then we get all the F_1 males white-eyed and all the F_1 females red-eyed.

This case is illustrated pictorially in Fig. 671.

Examples of sex-linked inheritance in man are colour-blindness and hæmophilia;

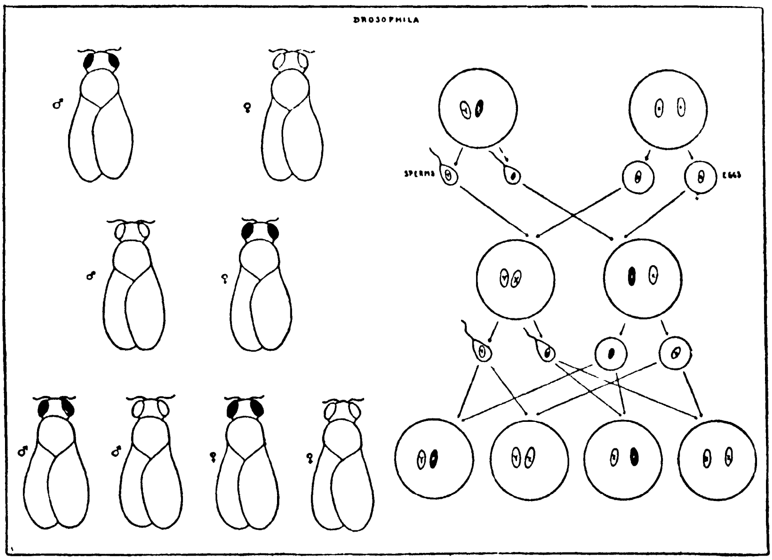

FIG. 671. Sex-linkage in *Drosophila*. Three successive generations at left: red eyes shown in black. The history of the sex-chromosomes through three generations shown at right. X-chromosome of original male in black. (SHARP, after MORGAN.)

these are borne by the X-chromosome and are recessive characters, hence they are carried by the female to a portion of the male descendants, but the females do not themselves suffer from the condition, unless, very exceptionally, both their parents were affected, or the father affected and the mother a carrier.

Linkage exists between various other pairs of characters, and the inference is that the genes concerned in the transmission of linked characters must be situated on the same chromosome, and hence be transferred together. The evidence for this, which is again chiefly derived from *Drosophila*, is that there are only as many independent factor-pairs as there are chromosomes in the gamete, or somatic chromosome-pairs. Hence, we conclude that the genes connected with a particular group of characters are located on a particular chromosome. From evidence derived from the detailed study of the phenomenon of crossing-over, it is further believed that for each gene is reserved a particular place in the chromosome, like each bead in a necklace, and that this position is faithfully retained in the ensuing generations.

Crossing-over. Although many phenomena of heredity are explicable on the supposition that the genes of a chromosome retain their positions relative to one another from generation to generation, thus giving rise to linkage, yet it appears that this does

not always apply as regards the two chromosomes of a somatic pair, carrying therefore similar, though not identical, genes. Let us suppose that, in such a pair, we have two series of genes ABCDEFG, etc., and abcdefg, etc. Then, when reduction division occurs in the formation of the gametes, it may happen that an interchange occurs, so that the gametes may be ABCdefg, etc., and abcDEFG, etc. (Fig. 672).

When this happens, although the linkage persists, there is a certain percentage of departures from the simple Mendelian law of independent association, and it is clear that the further apart on the chromosome any two genes are placed, the more likely will the exchange be to occur.

From the frequency with which this phenomenon of crossing-over happens for particular characters, their relative distance apart on the chromosome has been estimated, and gene-maps of the chromosomes deduced. Double crossing-over also occurs, *e.g* ABcdeFG, owing to twisting and cleavage having occurred twice in the process of reduc-

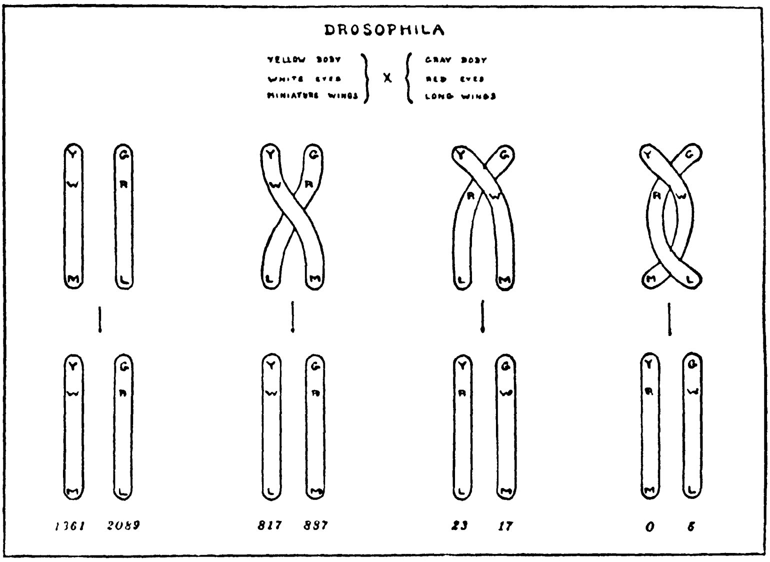

FIG. 672. Diagram to show Formation of New Linkage Groups by Single and Double Crossing-over. (From SHARP, after MORGAN.)

tion division. These interesting phenomena belong to the special subject of heredity and genetics and cannot be further discussed here.

Mutation. All breeders of animals and plants are aware that, occasionally, a new feature will suddenly appear and be transmitted thenceforward as an hereditary character. These spontaneously appearing characters are called mutations. On the basis of the gene hypothesis, mutation would be regarded as due to some stable alteration in the properties of the gene concerned. We are in complete ignorance as to how these mutations are brought about, so that we cannot, by any means known at present, produce a given mutation, though we can utilise those which occur spontaneously so as to retain the new quality in the breeding of subsequent generations. It has been shown that by the action of X-rays, mutations in *Drosophila* can be made to happen about 150 times as frequently as in nature, and this observation opens up further possibilities of exploring, and perhaps ultimately of controlling, this process.

In other instances, mutations have been shown to be associated with a change in the number of chromosomes: this occurs in some plants; thus Gates has found mutants of *Œnothera* with 15, 20, 21, 22, 23, 27, 28, 29 and 30 chromosomes.

The study of mutation has led to important modifications in our views as to the way in which, in the course of evolution, new forms have appeared. According to the principle of Natural Selection, as put forward by Darwin, any variations, if profitable, would be preserved by natural selection, and thus the process of evolution was a continuous one. The struggle for existence was an essential part of the hypothesis, since by its operation the less well endowed individuals were eliminated, and thus the swamping

of the profitable characters was avoided. We now know that mutants arise suddenly, and will breed true, so that swamping will not occur if the mutation is one which brings any biological advantage; the struggle for existence, though not essential for the production of new and better types, does lead to the loss of those types not able to compete.

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